論文の内容の要旨

論文題目　Contribution of Pancreatic Alpha Cell Function to Insulin Sensitivity and Glycemic Variability in Type 1 Diabetes
（ヒト１型糖尿病における膵α細胞予備能とインスリン抵抗性・血糖変動との関連性）

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Background

Type 1 diabetes mellitus is characterized by insulin deficiency caused by pancreatic beta cell destruction. Glucagon inappropriately secretes from pancreatic alpha cells and can exacerbate hyperglycemia due to paradoxical hyperglucagonemia or lead to severe hypoglycemia as a result of failed counter-regulation in patients with type 1 diabetes. Notably, patients with type 1 diabetes typically exhibit glucose metabolism mechanisms that are different from those of healthy individuals, including reduced insulin sensitivity, acutely increased blood glucose levels between 05:00 and 09:00 (called the “dawn phenomenon”), and impaired hepatic glucose uptake (HGU).

Despite extensive studies, the role of glucagon as a pathophysiologic factor remains unclear. In previous studies, glucagon levels were typically measured using conventional radioimmunoassay (RIA) kits. Quantitative assays known as sandwich enzyme-linked immunosorbent assays (ELISAs) have recently been developed.

Accordingly, in this study, we aimed to determine the contribution of pancreatic alpha cell function evaluated with RIA or ELISA kits to insulin sensitivity, HGU, and glycemic variability, including the dawn phenomenon, in patients with type 1 diabetes.

Materials and Methods

Study design and patients

This observational study was carried out at the National Center for Global Health and Medicine in Tokyo, Japan. We examined patients diagnosed with type 1 diabetes who met inclusion criteria and unmet exclusion criteria. Inclusion criteria were as follows: patients who were previously diagnosed with type 1 diabetes according to World Health Organization criteria and were at least 20 years of age. Exclusion criteria were as follows: current treatment with steroid hormones or immunosuppressants; pregnant or breastfeeding; estimated glomerular filtration rate (GFR) of less than 45 mL/min/1.73 m^2; current infection; and refusal to participate in the study.

Arginine stimulation test

Each patient underwent arginine stimulation tests to evaluate their pancreatic alpha cell function. Patients were asked to rest for 30 min after overnight fasting, and 30 g arginine was intravenously administrated over 30 min. Blood samples were collected before and 15, 30, 60, 90, and 120 min after arginine loading. The levels of plasma glucagon were measured by RIA and sandwich ELISA. The area under the concentration-time curve (AUC) of plasma glucagon between 0 and 120 min was calculated using the trapezoidal rule. The AUC of plasma glucagon measured by RIA kits was defined as AUC_{glcRIA}, and that measured by ELISA kits was defined as $AUC_{glcELISA}$. A peak glucagon level measured by RIA during arginine stimulation tests of equal to or more than 300 pg/mL was evaluated as glucagon hyperreactivity, whereas that of less than 300 pg/mL was evaluated as glucagon hyporeactivity.

Evaluation of changes in insulin requirements between night and morning as the “dawn phenomenon”

After the arginine stimulation test, we evaluated changes in insulin requirements between night and morning as the “dawn

phenomenon." At 20:00, two cannulas were placed in a forearm vein (for infusion of glucose and insulin) and in a heated contralateral forearm vein (for arterialized venous blood sampling) and then connected to an artificial pancreas. The artificial pancreas automatically primed insulin in accordance with an algorism to maintain blood glucose levels (80 to 110 mg/dL). The ratio of the delivered insulin dose average between 04:00 and 08:00 to that between 00:00 and 04:00 was calculated as the dawn index. To support the relationship between the dawn phenomenon and glucose-related hormones, we also measured levels of the following hormones after patients were kept at rest for 30 min after overnight fasting: growth hormone (GH); insulin-like growth factor-1 (IGF-1); adrenorcorticotropic hormone (ACTH); cortisol; active glucagon-related protein-1 (GLP-1); and somatostatin.

Hyperinsulinemic euglycemic clamp test

Hyperinsulinemic euglycemic clamp tests were applied to determine insulin sensitivity. At 8:00 after evaluating the dawn index, a primed-constant infusion of insulin was given at a rate of 2.58 mU/kg/min by the artificial pancreas to achieve a desired steady-state plasma insulin concentration (200 μU/mL). Subsequently, exogenous glucose infusion was initiated to maintain blood glucose levels within the euglycemic range (95 mg/dL) throughout the study and the exogenous glucose infusion rate (GIR; mg/kg/min) was adjusted by the artificial pancreas. The average of GIR during the last 90 min after achieving a steady-state was calculated as an indicator of the insulin sensitivity.

Multiple regression analysis was performed to examine the relationships between GIR as the dependent variable and the following independent variables: model 1 included age, sex, BMI, and AUC_{glcRIA}; model 2 included age, sex, BMI, and $AUC_{glcELISA}$.

Clamp oral glucose loading test

After hyperinsulinemic euglycemic clamp tests, clamp oral glucose loading tests were performed to evaluate HGU. Briefly, 90 min after the blood glucose concentration monitored by the artificial pancreas reached a steady-state level, a fixed amount of glucose (0.2 g/kg) was orally administered. GIR then started to decrease because some of the ingested glucose that was not extracted by the splanchnic tissues entered the systemic circulation and reduced the GIR required to maintain euglycemia. Subsequently, the GIR required to maintain euglycemia then returned to a normal level. We calculated HGU (%) using the following formula: HGU (%) = ([oral glucose load] – [GIR decrements]) / (oral glucose load). To support the relationship between HGU and glucose-related hormones, we also analyzed the correlation between HGU and fasting levels of GH, IGF-1, ACTH, cortisol, active GLP-1, and somatostatin.

Assessment of glycemic variability

Each patient underwent continuous glucose monitoring (CGM) for 96 h. The averages of the following variables over 3 days were calculated using the CGM data: mean blood glucose level, standard deviation (SD), M-value, mean amplitude of glycemic excursions (MAGE), hyperglycemic time, and hypoglycemic time. Hyperglycemic and hypoglycemic times were defined as the average number of minutes during which the patient's glucose levels were greater than 180 or less than 70 mg/dL in 1 day, respectively.

Results

Demographics

In total, 40 Japanese patients with type 1 diabetes who met the inclusion criteria and unmet the exclusion criteria participated in this study.

Glucagon response to arginine stimulation measured by RIA or ELISA

Correlations in the levels of plasma glucagon measured by RIA and ELISA at preloading and peak and those between logarithm-transformed AUC_{glcRIA} and $AUC_{glcELISA}$ were statistically significant (R^2 = 0.42, 0.25, and 0.20 and P = 0.001, 0.001, and 0.004, respectively). However, the levels of glucagon at preloading were undetectable by ELISA, even if those measured by RIA were detected in 17 of 40 (42.5%) patients. The peak levels and logarithm-transformed AUC levels of glucagon measured by RIA and ELISA were also decreased in some patients.

Associations between AUC_{glcRIA} or $AUC_{glcELISA}$ and the dawn index

The median (interquartile range) dawn index was 1.7 (1.0–2.8) and was not significantly correlated with AUC_{glcRIA} or $AUC_{glcELISA}$ (R^2 = 0.03, P = 0.39 and R^2 = 0.04, P = 0.32, respectively). We also analyzed the correlations between the dawn index and fasting levels of glucose-related hormones (i.e., GH, IGF-1, ACTH, cortisol, GLP-1, and somatostatin). There were no significant correlations among these parameters.

AUC_{glcRIA} and $AUC_{glcELISA}$ were associated with GIR, but not HGU, as evaluated by hyperinsulinemic euglycemic clamp and clamp oral glucose loading tests

The AUC_{glcRIA} and $AUC_{glcELISA}$ were significantly negatively correlated with GIR (R^2 = 0.20, P = 0.02 and R^2 = 0.21, P = 0.02, respectively) but not with HGU (R^2 = 0.07, P = 0.26 and R^2 = 0.01, P = 0.79, respectively). Considering confounding variables, multiple regression analysis demonstrated that none of the variables (age, sex, BMI, and AUC_{glcRIA}) were significant predictors of GIR in model 1. However, age and $AUC_{glcELISA}$ were significant predictors of GIR in model 2.

We also compared GIR, HGU, and baseline characteristics between patients with glucagon hypo- or hyperreactivity who could be evaluated GIR and HGU. Only GIR was significantly higher in patients with glucagon hyporeactivity than those with glucagon hyperreactivity (9.24 vs 6.75 mg/kg/min, P = 0.03).

We also analyzed correlations between HGU and fasting levels of glucose-related hormones. HGU was significantly correlated with fasting cortisol levels (R^2 = 0.28, P = 0.003) and was not correlated with any other glucose-related hormones.

AUC_{glcRIA}, but not $AUC_{glcELISA}$, was associated with glycemic variability evaluated by CGM

Of glycemic variability measurements, SD was significantly correlated with logarithm-transformed AUC_{glcRIA} positively (R^2 = 0.11, P = 0.049) but not with logarithm-transformed $AUC_{glcELISA}$ (R^2 = 0.01, P = 0.75). Other measurements were not significantly correlated with AUC_{glcRIA} or $AUC_{glcELISA}$.

Discussion

Glucagon (1–29) is produced through processing of proglucagon by proglucagon convertase. In this process, other proglucagon fragments (e.g., oxyntomodulin, glicentin, and GLP-1) were also produced. Measurement of glucagon with the RIA kit uses polyclonal antibodies against the glucagon C-terminal region, and these antibodies crossreact with other proglucagon fragments that also contain the C-terminal region. In contrast, double-sandwich ELISA kits use monoclonal antibodies against both the C- and N-terminal regions of glucagon and measure glucagon concentrations with much lower crossreactivity against proglucagon fragments other than glucagon (1–29). The discrepancy between AUC_{glcRIA} and $AUC_{glcELISA}$ in some patients suggested that the differences between responses of glucagon (1–29) to arginine stimulation and those of other proglucagon fragments.

In terms of associations between glucagon and insulin sensitivity, previous studies have shown that increased fasting levels of glucagon or glucagon responses to arginine stimulation can contribute to worsening insulin sensitivity in healthy individuals or patients with impaired glucose tolerance. The mechanisms through which alpha cells adapt to insulin sensitivity

remain unclear. The primary mechanism is thought to be “paracrinopathy,” which designates the loss of tonic restraint normally exerted by a high local concentration of insulin on alpha cells. In our study, GIR during hyperinsulinemic euglycemic clamp tests was significantly negatively correlated with AUC_{glcRIA} and $AUC_{glcELISA}$. Moreover, age and $AUC_{glcELISA}$ were independent variables for GIR in multiple regression analysis. These results suggested that pancreatic alpha cell function independently contributed to insulin sensitivity even in patients with type 1 diabetes, whose beta cell function is diminished.

The dawn phenomenon refers to the concept that the levels of blood glucose rise acutely between 04:00 and 08:00 and is typically observed in patients with type 1 diabetes. Circadian variations in counter-regulatory hormones (e.g., GH, IGF-1, and cortisol) could affect endogenous glucose production and cause the observed increase in blood glucose levels. In a study of healthy individuals, endogenous glucose production was found to increase as glucagon concentrations increased in the morning. However, subsequent studies concluded that there were no associations between the glucagon concentration and the dawn phenomenon. We also did not find any correlations between the dawn index and AUC_{glcRIA} or $AUC_{glcELISA}$. Thus, pancreatic alpha cell function appeared not to be related to the dawn phenomenon.

Cortisol has been shown to play a pivotal role in stimulation of HGU. The significant correlation between HGU evaluated by clamp oral glucose loading tests and fasting levels of cortisol in our study appeared to indicate the pathophysiological effects of cortisol on hepatic glucose metabolism. Interestingly, hepatic glucose production is rapidly stimulated by the physiological rise in glucagon, which is entirely attributable to enhancement of glycogenolysis. A previous report in patients with insulin-dependent diabetes could not find any association between glucagon response to oral glucose loading and HGU. In the present study, we also did not find any significant correlations between HGU and AUC_{glcRIA} or $AUC_{glcELISA}$. These results indicated that pancreatic alpha cell function was not associated with HGU in patients with type 1 diabetes.

In terms of glycemic variability, our study showed that AUC_{glcRIA} was significantly correlated with the SD of glucose levels but $AUC_{glcELISA}$ was not correlated with the measurement of glycemic variability. The discrepancy between the correlation of glycemic variability with AUC_{glcRIA} and $AUC_{glcELISA}$ in the present study appeared to indicate that proglucagon fragments other than glucagon (1-29) could contribute to glycemic variability.

In conclusion, we found that pancreatic alpha cell function contributed to insulin sensitivity but did not affect HGU and glycemic variability including the dawn phenomenon, in patients with type 1 diabetes. The relationships between pancreatic alpha cell function and glycemic variability could be affected by the purity of glucagon assays. These data provide important context for the multifactorial role of glucagon in glucose metabolism in patients with type 1 diabetes.