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Original Article (4th revision)

Postprandial serum C-peptide value is the optimal index to identify patients with non-obese type 2 diabetes who require multiple daily insulin injection: analysis of C-peptide values before and after short-term intensive insulin therapy

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ABSTRACT

Aims/Introduction: Type 2 diabetes is a progressive disease characterized by a yearly decline in insulin secretion; however, no definitive evidence exists indicating the relationship between decreased insulin secretion and insulin treatment regimen. To determine the optimal insulin secretory index for identifying patients with non-obese type 2 diabetes who require multiple daily insulin injection (MDI), we evaluated various serum C-peptide immunoreactivity (CPR) values.

Materials and Methods: We near-normalized blood glucose with intensive insulin therapy (IIT) over a 2-week period in 291 patients with non-obese type 2 diabetes, based on our treatment protocol. After improving hyperglycemia, we challenged with oral hypoglycemic agent (OHA), and according to the responsiveness to OHA, patients were classified into 3 therapy groups: OHA alone (n=103), basal insulin plus OHA (basal insulin-supported oral therapy, BOT) (n=56), and MDI (n=132). Glucagon-loading CPR increment (ΔCPR), fasting CPR (FCPR), CPR 2 hours after breakfast (CPR2h), the ratio of FCPR to FPG (CPI), CPI 2 hours after breakfast (CPI2h), and secretory unit of islets in transplantation (SUIT) were submitted for the analyses. ROC and multiple logistic analyses for these CPR indices were carried out.

Results: Many of the CPR values were significantly lower in the MDI compared with the OHA alone or BOT groups. ROC and multiple logistic analyses disclosed that post-prandial CPR indices (CPR2h and CPI2h) were the most reliable CPR markers to identify patients requiring MDI.

Conclusions: Postprandial CPR level after breakfast is the most useful index for identifying patients with non-obese type 2 diabetes who require MDI therapy.

(249 words)

KEY WORDS: C-peptide, Meal load, Multiple daily insulin injection

INTRODUCTION

Type 2 diabetes mellitus has been shown to be a progressive disease that is characterized by a yearly decline in insulin secretion[1,2,3]. For chronic stage type 2 diabetes, insulin therapy can involve various regimens including basal insulin-supported oral therapy (BOT) or multiple daily insulin injection (MDI). The American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) have published a consensus statement[4] regarding the management of hyperglycemia in type 2 diabetes. This includes a practical algorithm of the therapy, based on blood glucose and HbA1c, which progresses from oral hypoglycemic agent (OHA) to basal insulin therapy, and then to MDI. The progression from OHA to MDI in type 2 diabetes is assumed to be closely related to the decrease in insulin secretory capacity. Although, in the daily practical diabetes care, whether insulin therapy for stable glycemic controls is required or not is a significant issue for patients and physicians, no useful insulin secretory index for commencing insulin therapy have existed. Recently, regarding serum C-peptide immunoreactivity (CPR) as a marker for predicting insulin requirement in type 2 diabetes, several reports have been published[5,6,7], where useful CPR indices were advocated. In this study, to determine the optimal CPR index for identifying MDI-requiring patients with non-obese type 2 diabetes, we analyzed retrospectively various serum CPR values by comparing the values among different diabetes therapy groups, which were determined according to our treatment protocol. The protocol consists of intensive insulin therapy (IIT) and challenge of OHA mainly including insulin secretagogues after IIT.

MATERIAL AND METHODS

Patients

Using our department diabetes database, we initially selected 1039 patients with type 2 diabetes who had been hospitalized and treated with insulin for poor glycemic control, over a 36-month period between October 2007 and September 2010. Among this group, those with incomplete plasma glucose (PG) values (163 patients) or CPR (89 patients), or those in a preoperative state (109 patients) were excluded, leaving 678 patients. Then, another 109 patients with conditions influencing CPR assessment or selection of insulin therapy, including those with chronic liver disease (37), malignancies (32), dementia (13), acute infections (11), diabetic foot (8), or who deviated from the treatment protocol (8), were also excluded from the study, leaving 569 patients. Of these, 291 non-obese (body mass index, BMI of less than 25) patients with type 2 diabetes were enrolled in the study.

The mode of treatment at baseline in these cases was OHA alone in 160 patients (a sulfonylurea in 115), insulin in 62 patients (combined with OHA in 21), and no medical

treatment in 69 patients. Table 1 shows the baseline clinical characteristics and type of treatment at enrollment in these patients.

Treatment protocol and its details

Treatment proceeded based on a 2-week treatment protocol. On day 1, a standard diabetes meal, 30 kcal/kg of standard body weight: 22×body height $(m)^2$, consisting of 62% carbohydrate, 16% protein, and 22% fat (when taking 1600 kcal diet per day), was started. Patient self-measurement of capillary blood glucose 4 times daily (before each meal and at bedtime) by using a portable blood glucose monitor (Glutest Ace R, Sanwa Kagaku Co. Ltd., Nagoya, Japan) was also started. On day 2, PG excursion at 7 points (before 3 meals, 2 hours after starting meals, and at 10 PM) was measured under the baseline treatment of OHAs or insulin injections. On the morning of day 3, previous treatments were discontinued and a glucagon-loading test was performed. Thereafter, IIT was started as described below.

In all patients, injections before each meal with 4 U of regular or ultra short-acting analogue insulin, and at 10 PM with 4 U of intermediate-acting insulin (NPH), insulin glargine, or insulin detemir were started. The insulin dose was adjusted daily for a target glucose value of 110 mg/dl before the 3 meals.

On days 8-9, insulin was discontinued and OHA was started, consisting of either 40-80 mg gliclazide, 1-2 mg glimepiride, 270 mg nateglinide, or 30 mg mitiglinide in combination with either 150-300 mg acarbose, 0.9 mg voglibose, or 150 mg miglitol (daily doses of each). These medicines were administered for 3 days, and if glucose values were equal to the values obtained with IIT, the OHA treatment was continued. If glucose was ≥140 mg/dl before breakfast, the original bedtime insulin (same type and dose at 10 PM) was added. If glucose was ≥140 mg/dl both before breakfast and before dinner, all OHAs were discontinued, and a biphasic analogue-mixed insulin, at 80% of the total daily insulin dose prior to switching, split in a 2:1 ratio, was started before breakfast and before dinner. When, despite 2 injections of the insulin, appropriate blood glucose levels were not achieved, either one-half of the morning dose of the same insulin was added as a 3rd injection before lunch, or the 4 times daily insulin regimen used before switching was resumed. On day 13, PG excursion was measured again, and final adjustments to OHAs or insulin dose were made. According to the protocol, the final treatment regimen was divided into 3 groups: OHA alone, basal insulin plus OHA (BOT), and insulin 2-4 times daily (MDI).

Patients previously admitted to our hospital, and who were at that time assessed as requiring MDI, or in whom 2 physicians, including a diabetologist certified by the Japan

Diabetes Association, judged MDI necessary were continued on insulin therapy without OHA challenge.

PG and CPR sampling

On day 2, PG (mg/ml) excursion was measured at 7 points. At that time, fasting CPR (FCPR) (ng/ml) before breakfast and CPR 2 hours after starting the meal (CPR2h) were measured. On day 3, under fasting conditions, an intravenous glucagon (1 mg) loading test was performed, and CPR was measured at 0 min and 6 min. On day 13, PG excursion, FCPR and CPR2h were also measured.

The CPR indices submitted for analysis included the glucagon loading CPR increment (ΔCPR) on day 3, FCPR, CPR2h, the ratio of FCPR to fasting plasma glucose (FPG): C-peptide index (CPI, FCPR/FPG × 100, ng/mg)[7], CPI2h (CPI 2 hours after breakfast), and the secretory units of islets in transplantation (SUIT, %)[8], which was calculated by the formula: 1485 × FCPR/ (FPG – 61.8)[8], on day 2 and day 13.

CPR was measured by radioimmunoassay (RIA) using a C-Peptide Kit "Daiichi" III (TFB Inc., Tokyo, Japan). Seven points of daily PG excursion values before starting and after completing IIT were determined in venous blood by the hexokinase method.

Statistical analysis

The clinical characteristics of subjects used in the analysis were age, period from diagnosis of diabetes (disease period), BMI, FPG, HbA1c (National Glycohemoglobin Standardization Program, NGSP value), serum CPR concentrations, and calculated CPR values. Among the OHA alone, BOT, and MDI groups, clinical markers and individual CPR values were analyzed using ANOVA. Also, inter-group differences between the OHA group (defined as the OHA alone group combined with the BOT group) and MDI group were analyzed using a non-paired t-test. In addition, to uncover indices capable of discriminating the requirement for MDI treatment from that of other treatments, ROC and multiple logistic regression analyses of each CPR index were performed. Contribution of disease period to necessity of MDI was tested with ROC analysis as well. The statistical software used for analyses were Excel Statistics 2010 for Windows ver. 1.09 (Social Survey Research Information Co. Ltd., Tokyo, Japan) and IBM SPSS Statistics ver. 19 (IBM Japan, Tokyo, Japan). HbA1c values were converted from JDS to NGSP values by the conversion equation[9].

The clinical study and treatment protocol were submitted to the Clinical Research Approval Committee and approved by the Medical Ethics Committee of Kurashiki

Central Hospital. Before initiation of treatment, the attending physician provided a written explanation of the study protocol and verbal consent was obtained from all patients.

RESULTS

Daily plasma glucose excursions before and after IIT

Under the baseline treatment conditions before starting IIT, the daily PG excursion values at the 7 points were 184±59, 293±95, 253±94, 264±109, 200±84, 266±89, and 252±85 mg/dl (mean±SD) in all subjects. Under the final treatment conditions assigned after IIT, values were 117±22, 174±52, 142±42, 175±54, 135±44, 169±54, and 162±51 mg/dl (mean±SD). Significant decreases were observed at all points ($p<0.01$).

Clinical characteristics and CPR levels according to treatment groups

Of the 291 patients, the number in each final therapy was: OHA alone, 103; BOT, 56; and MDI, 132 (2 insulin injections per day, 95; 3 injections, 15; and 4 injections, 22). The relationships between baseline treatment and each final therapy are shown in Figure S1.

Details of oral agents used in the OHA alone group are shown in Table S1a, and details of oral agents combined with insulin and basal insulin remedies in the BOT group are shown in Table S1b. Details of insulin therapy modes in the MDI group are shown in Table S1c.

Table 2 shows the baseline clinical characteristics in the 3 groups, including the following 6 indices: FCPR, ΔCPR, CPR2h, CPI, CPI2h, and SUIT. ANOVA analysis among the 3 groups showed that, in the MDI group, period from diagnosis was longer and BMI was smaller compared with the OHA alone and/or BOT group ($p<0.01$), whereas HbA1c levels were not different among the groups. Analysis of CPR values revealed no significant differences between the OHA alone and BOT groups, while all CPR values were significantly lower in the MDI group ($p<0.01$; $p<0.05$, in SUIT) than in the OHA alone group, except for FCPR. CPI and SUIT did not significantly differ in the BOT and MDI groups, whereas all other values were lower in the MDI group ($p<0.01$; $p<0.05$, in FCPR).

Because no differences were observed in any of the CPR indices between the OHA alone and BOT groups, and because both groups were responsive to OHAs, they were combined to form the OHA group. As shown in Table 3, a comparison of the clinical characteristics and CPR values between the OHA and MDI groups revealed that period from diagnosis was longer, BMI was smaller, and all CPR values before IIT ($p<0.01$; $p<0.05$, in SUIT) and after IIT ($p<0.01$) were lower in the MDI group compared to the

OHA group.

ROC analysis of CPR indices to determine the requirement for MDI before and after IIT

In ROC analysis of CPR indices using the baseline data before IIT, as shown in Table 4a, the AUC and specificity were: ΔCPR, 0.742 and 69.2% (cut-off, 1.5 ng/ml); CPR2h, 0.752 and 82.4% (3.0 ng/ml); CPI, 0.692 and 68.6% (0.6 ng/mg); CPI2h, 0.779 and 79.2% (1.0 ng/mg); SUIT, 0.677 and 62.3% (15%), respectively. AUC and specificity were higher in both CPR2h and CPI2h compared to the other 3 indices. Sensitivity for all indices was about 60%. In ROC analysis of the data after IIT, as shown in Table 4b, the AUC and specificity were: CPR2h, 0.902 and 86.8% (cut-off, 3.0 ng/ml); CPI, 0.811 and 81.8% (0.6 ng/mg); CPI2h, 0.912 and 76.1% (2.0 ng/mg); and SUIT, 0.807 and 83.0% (20%), respectively. Although almost all indices showed increases in AUC, sensitivity, or specificity compared to baseline, CPR2h and CPI2h were still superior to the others. Figure S2a and S2b show the ROC curves of these indices. Disease period contributed to identifying patients requiring MDI (AUC 0.730, sensitivity 52.8%, and specificity 77.0% at cut-off of 15 years), however, the AUC and specificity were lower compared to that of the main CPR indices (Table 4a).

Multiple logistic analysis of CPR indices to determine the requirement for MDI before and after IIT

Multiple logistic analysis of CPR indices before and after IIT to determine the requirement for MDI therapy was performed. The summarized results are shown in Table 5a and 5b. In order to avoid multicollinearities lying between CPR indices, each CPR was separately analyzed together with the clinical markers BMI and disease period in common (refer to Table S2a and S2b). Of the 5 CPR parameters before IIT, ΔCPR, CPR2h, CPI, and CPI2h were selected as significant explanatory variables; however, a standardized partial regression coefficient was advantageous in ΔCPR, CPR2h, and especially CPI2h. Of the 4 CPR parameters after IIT, all were selected as significant explanatory variables; however, a standardized partial regression coefficient was advantageous in CPR2h and CPI2h.

DISCUSSION

In this study, statistical analyses disclosed that the CPR indices that best discriminated the requirement for MDI from the other treatments were CPR2h and CPI2h. Patients of the MDI group had a longer diabetes duration, were lean, and characteristically had lower

baseline CPR levels, when comparing clinical profiles and CPR levels in 3 patient groups which were assigned after IIT: OHA alone, BOT, or MDI. A limitation of this study might be that the final treatment regimen was determined in 3 days. Nevertheless, our study design in which after near-normalization with IIT under a strict diet, rapidly effective oral agents, such as sulfonylurea (SU) or glinide, were used[4] could determine the CPR indices contributed to distinguishing the 3 therapy groups, and to identifying patients who required MDI.

The reasons we targeted type 2 diabetes with a BMI of less than 25 in present study are that although obesity has recently been increasing in the Japanese, they have traditionally been non-obese, and that because a large BMI and liver insulin resistance influences daily insulin requirement in type 2 diabetes[10], obesity might lead to bias in treatment selection. Enrollment of only for non-obese patients, however, would narrow the applicability of our results for clinical use. Therefore, further analysis of the treatment for obese compared to non-obese type 2 diabetes will be needed.

CPR indices judged to be the most useful for MDI therapy were the postprandial CPR levels; total CPR concentration after meal consists of postprandial glucose-stimulated insulin secretion and glucose-dependent insulin secretion by incretin[11]. Of these insulin secretion mechanisms, regarding the latter, Bagger et al.[12] recently reported that the regulation of incretin effect was impaired in patients with type 2 diabetes. Advantage of indices CPR2h and CPI2h, which were obtained with the physiological meal load unlike the other indices, may have reflected dysfunction of these two mechanisms. Although in patients with advanced stage diabetes such as the MDI group, whether or not the incretin effect further decreases remains to be elucidated[30].

Funakoshi et al.[6] performed ROC analysis of CPR values as indices indicative of insulin therapy in type 2 diabetes, and found CPI to be superior among several CPR markers. They noted that because CPI could be calculated solely from a one-point blood sample, it was convenient and less burdensome. In their study, however, CPR2h and CPI2h were not provided as CPR indices. Saisho et al.[5] reported that postprandial CPR to plasma glucose ratio was the best predictor of subsequent insulin treatment in type 2 diabetes. Although their method that determined requirement for insulin therapy was different from ours, the usefulness of postprandial CPI agreed with our results in present study. Meier et al.[13] analyzed the relationship between CPR indices and human pancreatic β-cell area (determined from surgical specimens); in comparison to fasting measures such as CPI, CPI 15 min or 30 min after oral glucose loading showed better correlation with β-cell area. This shows that the postprandial CPI plays a significant role. Funakoshi et al.[14] compared postprandial CPR (PPCPR) to glucagon-loading CPR (CPR6min) in type

2 diabetes, and showed that PPCPR level was influenced by chronic hyperglycemia (estimated with HbA1c) to a greater extent than CPR6min level, and was more subject to glucose toxicity than CPR6min or FCPR. These results are interesting for us, considering the improved utility of CPR2h after IIT in present study. While glucagon loading is a non-physiological test, although ΔCPR value obtained from this test is one of the confirmed indices estimating a yearly decline of endogenous insulin secretion[15], utility of the value as an indicator for MDI was inferior to the postprandial indices as showed by present ROC analysis.

By near-normalization of blood glucose with IIT, a diminution of glucose toxicity and recovery of pancreatic β-cell function may be expected[16,17]. In the MDI group, however, baseline CPR levels were originally low, and even with IIT CPR levels remained low (Table 3), whereas in the OHA group all CPR levels, except for FCPR, were elevated (statistical analysis was not performed because this point was not within the scope of the work). As shown in Table 4a and 4b, ROC analysis showed that after IIT, compared to before IIT, the AUC of each CPR index increased. This was presumed to contribute to the recovery of CPR levels after ITT in the OHA group, but not in the MDI group.

Similarities in all measured CPR levels were observed in the OHA alone and BOT groups, and both groups were responsive to OHAs. Therefore, the OHA alone and BOT groups were combined to form the OHA group. Even though CPR levels were similar in the 2 groups, basal insulin injection was required in the BOT group. The reason is because BMI and baseline FPG were significantly higher in the group as shown in Table 2, and because liver insulin resistance is one of the main pathophysiological features in the obese patients; therefore, basal insulin injection was required to suppress hepatic glucose output (HGO) [18] in the somewhat heavier BOT group. Combination therapy with basal insulin plus oral agents using bedtime NPH insulin and daytime SU originated in North America and Northern Europe[18,19,20], and the clinical utility of this regimen has been demonstrated[21,22]. Currently, the long-acting analogue insulin glargine or detemir is used as basal insulin because of the convenience and efficacy, and the combination therapy with OHA is termed BOT. However, the clinical characteristics and insulin secretory ability of type 2 diabetics responding positively to BOT have not been thoroughly investigated[19]. Our results showed that insulin secretion in patients assigned to BOT was clearly sustained compared to the MDI group, and was similar to the OHA alone group. The clinical marker distinguishing the BOT group from the OHA alone group was not CPR, but rather BMI and FPG.

Incretin-related agents, such as dipeptidyl peptidase-4 (DPP-4) inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonists, have recently been introduced;

however, these agents were not an option in the treatment protocol of the present study. Incretin agents, unlike other antidiabetic agents, exert GLP-1 effects on glucose-dependent insulin secretion and pancreatic β-cell protection[23], and thus may influence selection for insulin therapy in type 2 diabetes. Kozawa et al.[24] reported, however, that patients with decreased insulin secretion showed lowered efficacy of GLP-1 receptor agonist liraglutide. An exploring study[25] using another GLP-1 receptor agonist exenatide showed that insulin-treated type 2 diabetes were deteriorated in glucose control in 38% of the patients assigned to exenatide by switching insulin to that. These studies suggest that cautions are needed for switching to injection of a GLP-1 receptor agonist in such as MDI-requiring patients. Administration of a DPP-4 inhibitor can enhance the action of SU; therefore, in BOT patients taking SU, another treatment option may be possible. In a 24-week study of the effects of 100 mg sitagliptin co-administration on insulin in type 2 diabetic patients[26], FPG and HbA1c were significantly improved; however, the total daily insulin dose did not change, nor occurred insulin elimination.

In the present study, we reported the optimal CPR indices, as well as their cut-off values, for determining the need for insulin therapy. However, according to the results of ROC analysis, as shown in Table 4a and 4b, the sensitivity and specificity of the CPR indices was 60 to 80% at a cut-off value, which might not always be practical. Therefore, caution is advised in basing the need for insulin therapy in any given patient solely on CPR values. Because of the difficulty in routinely estimating insulin sensitivity at the bedside[27], the evaluation was not included in this work. However, reports have indicated that even with IIT, insulin sensitivity was only partially reversed[16], or was not improved[28]; accordingly, the present data suggests that pancreatic β-cell dysfunction contributes most to the selection of treatment regimen.

Some discrepancies existed between our results and those of other reports[5,6,7] in baseline CPR levels and cut-off values of CPR indices for MDI in ROC analysis. Lower FCPR and CPI levels at baseline in our study were mainly caused by lower average BMI of the subjects, and lower CPI cut-off value might be attributable to the methodology in determining insulin requirements, which was different from that of other reports.

In the MDI group, as shown in Table S3, serum creatinine concentration was higher, and creatinine clearance was lower compared to the OHA alone and BOT groups ($p<0.05$). As renal dysfunction affects CPR excretion from the kidneys, this could elevate serum FCPR and CPR2h concentrations; therefore, presence of the slight renal dysfunction might have rather underestimated the usefulness of CPR index for MDI mainly by lowering the sensitivities at the cut-off values of postprandial CPR indices. Incremental CPR by meal load (not included in this study) could be more useful in

patients with renal dysfunction to estimate insulin secretory ability.

In a review, Yagihashi asks[29], "What determines the insulin requirement in type 2 diabetes mellitus?" and "Are all patients who require insulin severely diabetic or in the advanced stage?", thereby advocating the need for clinical staging of type 2 diabetes. It is likely that measurement of some CPR index could be an accurate marker for both setting up the staging and determining the severity of type 2 diabetes, as well as an index to determine a treatment regimen for diabetes.

In conclusion, in patients with non-obese chronic stage type 2 diabetes, postprandial serum CPR value measured at 2 hours after breakfast is the optimal CPR index to identify patients requiring MDI. Follow-up evaluation of the selected therapy regimen would confirm our results; as well as, another study analyzing CPR indices for MDI therapy in obese type 2 diabetes is required.

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(3978 words)

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Flow schema from baseline treatment to the final stage therapy.