Improved post-prandial ghrelin response by nateglinide or acarbose therapy contributes to glucose stability in Type 2 diabetic patients

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ABSTRACT. Background: Recent studies highlight an important role of ghrelin in glucose homeostasis, while the association between ghrelin regulation and glucose fluctuation is unclear. Aim: We compared the effects of two postprandial hypoglycemic agents on ghrelin response and determined the contribution of ghrelin response to glucose stability in Type 2 diabetic (T2DM) patients. Subjects and methods: Forty newly-diagnosed T2DM patients were randomly allocated to receive nateglinide or acarbose for 4 weeks, with twenty body mass index (BMI)-matched normoglycemic subjects as controls. Mean glucose values and daily average glucose excursion were assessed using continuous glucose monitoring system. Serum ghrelin levels were determined by enzyme-linked immunosorbent assay. Results: T2DM patients had similar fasting ghrelin levels (p=0.546), while their postprandial ghrelin suppressions at 30 min and 120 min were reduced as compared to BMI-matched normoglycemic controls (p<0.01). Both nateglinide and acarbose increased post-prandial ghrelin suppression at 120 min and reduced ghrelin area under the curve (AUCghRL) (p<0.05), while only nateglinide increased post-prandial ghrelin suppression at 30 min (p<0.01), which was positively correlated with the increased early-phase insulin secretion by 4 weeks of nateglinide therapy (r=0.48, p=0.05). The decrease in AUCghRL was positively correlated with the decrease in daily average glucose excursion and mean glucose values either by 4 weeks of nateglinide or acarbose therapy (p<0.05). Conclusions: Both nateglinide and acarbose increase post-prandial ghrelin suppression. Improved ghrelin regulation is most likely to play a role in glucose stability in T2DM patients with nateglinide or acarbose therapy. (J. Endocrinol. Invest. 36: 489-496, 2013)

INTRODUCTION

Increasing evidence suggests that acute glucose fluctuation around a mean value over a daily period, by activating the oxidative stress, might play an important role in the pathogenesis of diabetic complication. As a consequence, it is strongly advised that a global anti-diabetic strategy should be aimed at reducing the different components of dysglycemia [glycated hemoglobin (HbA1c), fasting and post-prandial glucose levels, glucose variability] (1, 2). Currently, the daily mean blood glucose values (MBG) and daily glucose fluctuation can be effectively assessed in humans using a continuous glucose monitoring system (CGMS), and the mean amplitude of glycemic excursions (MAGE) is commonly accepted as a gold standard for the assessment of daily glycemic variability (3, 4). Defect in insulin secretion is the major factor leading to sustained hyperglycemia and glucose fluctuation in Type 2 diabetes (T2DM) (5).

Ghrelin, a novel 28-amino acid peptide produced primarily by the stomach and proximal small intestine, has been found to inhibit insulin secretion both in vivo and in vitro studies (6, 7). Despite the rising evidence of ghrelin involved in glucose metabolism, no study has examined the correlation between the ghrelin regulation and acute glucose fluctuation. Circulating concentrations of ghrelin typically increase on fasting and decrease following food intake (8). Though ghrelin dysregulation has been found in obese subjects, only a few studies investigated ghrelin regulation in T2DM with controversial results (9, 10). Moreover, data about the effect of individual anti-diabetic agents on ghrelin regulation in T2DM is limited, though it has been shown that metformin may prolong a post-prandial fall in ghrelin, while the other insulin-sensitizing agent, pioglitazone had no effect on circulating ghrelin levels, coincident with a prolonged sensation of fullness and suppression of metformin therapy-induced hunger (11, 12).

The anti-diabetic agents which potentially target post-prandial hyperglycemia in T2DM include glinide drugs and α-glucosidase inhibitors. The D-phenylalanine derivative nateglinide acts as an insulin secretagogue with a rapid and short duration of action, to restore early-phase insulin response, and thus suppresses post-prandial hyperglycemia in T2DM (13). Acarbose, an α-glucosidase inhibitor, reduces or delays carbohydrate digestion by competitive inhibition of α-glucosidase enzymes in the brush border of the small intestine (14). Both nateglinide and acarbose are effective in T2DM for post-prandial glucose fluctuation (15), though stimulating insulin secretion from pancreas, glinide gained less weight in comparison to sulfonylureas, and acarbose had neutral effect on body weight (16, 17). Bearing in mind the effect of ghrelin on body weight gain and glucose metabolism, the relative
advantages of nateglinide and acarbose on body weight and improvement of glucose stability may be associated with ghrelin response in T2DM.

In this open-label, randomized, and prospective study, we compared the effects of nateglinide and acarbose mono-therapy on ghrelin response in T2DM patients and explored the contribution of the improved ghrelin regulation to glucose stability after 4 weeks of nateglinide or acarbose therapy.

SUBJECTS AND METHODS

Study design

The study protocol was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects, with local Ethics Committee approval. Forty patients (18-65 yr, 25 males and 15 females) with newly diagnosed T2DM and BMI<30 kg/m² were recruited from the Sir Run Run Shaw Hospital endocrine clinic. Diagnosis and classification of diabetes were performed according to the World Health Organization criteria of 1999. Body weight, height, waist circumference (WC) and blood pressure were measured using standardized methods. All patients were naïve to oral anti-diabetic agents, and they had HbA₁c levels of 6.5-9.0%. They were advised to comply with diabetes self-management including total calories restriction (approximately 25-30 kcal/kg-d of standard body weight), distribution of total calories [breakfast 20%, lunch 40%, dinner 40%] and nutritional components (60 carbohydrate energy % (En%), 25 fat En% and 15 protein En%) over 3 meals, appreciated amounts of exercises and habitual bedtimes from 22:00-24:00 h until 06:00-08.00 h. They were also instructed to consume each meal within half an hour with fixed feeding schedules (breakfast 06:00-07.30 h, lunch 11:30-13.00 h, and dinner 18:00-19:30 h) and to record any additional food-intake during the entire period of the study. After one week of diabetic dietary therapy, patients were randomized (1:1) to receive 120 mg nateglinide (no.=20) or 50 mg acarbose (no.=20) three times a day for 4 weeks. A 72 h CGMS monitoring and meal test were carried out before the start of drug therapy and at the end of therapy. Twenty BMI-, age- and sex-matched controls with no family history of diabetes were selected on the basis of the Oral Glucose Tolerance Test results [fasting blood glucose (FBG) <6.1 mmol/l and 2-h post-prandial blood glucose (2h PBG) <7.8 mmol/l]. Non-diabetic controls were also asked to conduct the same diet- and exercise-based strategies 1 week before the start date of the study, including the 3 day CGMS monitoring period, in accordance to the same principles as the T2DM patients.

Exclusion criteria for all subjects included: impaired renal or liver function, a history of angina pectoris, cerebral or myocardial infarction, and treatment with angiotensin converting enzyme inhibitors, angiotensin receptor blockers, statins, aspirin, glucocorticoid therapy or thyroid hormone therapy.

CGMS monitoring

T2DM patients underwent up to 72 h CGMS monitoring at baseline (after one week of diabetic dietary therapy and 3 days before the initiation of drug therapy) and at the end of drug therapy. Control subjects underwent 72 h CGMS monitoring once. During the 72 h CGMS monitoring period, at least four daily self-monitoring capillary blood glucose measurements, obtained with a glucometer (OneTouch® Ultra®, Johnson & Johnson, NJ, USA), were entered into the CGMS monitor (CGMS, Medtronic MiniMed, Northridge, CA, USA) for calibration. After three days monitoring, all subjects returned to the hospital, the CGMS data was downloaded via the Com-Station using the MiniMed Solutions Software version 3.0 (MiniMed) and the 24 h glucose profile for each of the 3 days was analyzed.

Assessment of glycemic instability

The mean blood glucose value (MBG) was calculated as the arithmetic mean glucose value over a 24 h period. To assess intra-day glycemic variability, the mean amplitude of glycemic excursion (MAGE) was determined, using a MAGE algorithm to calculate the average peaks and nadirs with an amplitude >1 SD of mean glucose over a 24 h period, averaged over a continuous monitoring period of 48 h (4).

Meal test

The subjects were asked to return to the hospital on the last morning of the 72 h CGMS monitoring period after a 12 h overnight fast to receive a meal test. Subjects were instructed to refrain from smoking and drinking tea, coffee or alcoholic beverages the night before the study. The test meal was semi-liquid and consisted of 71.0 g carbohydrate, 11.0 g fat and 8.2 g protein containing a total of 416 kcal, and had to be consumed within 5 min. At the end of the study, all T2DM patients were asked to take nateglinide or acarbose before or with the test meal. Blood samples were collected at fasting, 30 min and 120 min after ingestion.

Sample collection and laboratory measurements

Fasting, 30 min and 120 min plasma glucose concentrations during the test meal were measured using enzymatic methods. Total glucose area under the curve (AUCGLU) was calculated as 0.25 × [FBG + 4 × 30 min PBG + 3 × 120 min PBG]. Total cholesterol (TC), triglycerides (TG), serum creatinine (Scr) and alanine aminotransferase (ALT) were analyzed in fasting blood samples using enzymatic techniques. HDL cholesterol (HDL-C) concentration was quantified using polyethylene glycol-modified enzymes, and LDL cholesterol (LDL-C) concentration was calculated using the Friedewald formula. HbA₁c was measured by fast performance liquid chromatography (Bio-Rad) and serum glycated albumin (GA) by a liquid enzymatic method using a Lucci® GA-L kit (Sekisui Medical Co. Ltd, Tokyo, Japan) on an automatic biochemical analyzer Glamour 2000. Serum fasting (FINS), 30 min (30 min INS) and 120 min (120 min INS) insulin levels were measured using an immunoassay. Insulin resistance was assessed from the fasting blood glucose and insulin levels, using homeostasis model assessment of insulin resistance (HOMA-IR) according to the following formula: FBG (mmol/l) × (FINS + 4 × 30 min PBG + 3 × 120 min PBG). Fasting, 30 min and 120 min insulin levels were measured using an immunoassay. Insulin resistance was assessed from the fasting blood glucose and insulin levels, using homeostasis model assessment of insulin resistance (HOMA-IR) according to the following formula: FBG (mmol/l) × (FINS + 4 × 30 min PBG + 3 × 120 min PBG). The separated serum was stored at −70 C until ghrelin analysis.

ELISA assay for serum ghrelin (total) levels

Total serum ghrelin levels at fasting, 30 min and 120 min after the meal were determined using an enzyme-linked immunosorbent assay kit (Millipore, Billerica, MA, USA). The intra-assay coefficient of variation (CV) was 1.0% and inter-assay variation was