Clinical application of ghrelin for diabetic peripheral neuropathy

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Abstract. Diabetic peripheral neuropathy (DPN) is the most common complication of diabetes, and its progression significantly worsens the patient’s quality of life. Although several drugs are available for DPN, all of these provide only symptomatic relief. We investigated the therapeutic effects of ghrelin for DPN, based on its various physiological functions. Seven patients with type 2 diabetes with typical clinical signs and symptoms of DPN were hospitalized. Synthetic human ghrelin (1.0 µg/kg) was administered intravenously for 14 days. Motor nerve conduction velocity (MCV) of the posterior tibial nerve improved significantly after the treatment, compared to that at baseline (35.1 ± 1.8 to 38.6 ± 1.8 m/s, \( p < 0.0001 \)), while the MCV in six untreated patients did not change throughout hospitalization. The subjective symptoms assessed based on the total symptom score also significantly improved (15.6 ± 3.1 to 11.1 ± 2.2, \( p = 0.047 \)). Although sensory nerve conduction velocity (SCV) of the sural nerve could not be detected in three patients at baseline, it was detected in two of the three patients after 14 days of ghrelin administration. Overall, SCV did not change significantly. Plasma glucose, but not serum C peptide, levels during a liquid meal tolerance test significantly improved after treatment. These results suggest that ghrelin may be a novel therapeutic option for DPN; however, a double-blind, placebo-controlled trial is needed in the future.

Key words: Ghrelin, Diabetic neuropathy, Motor nerve conduction velocity

Material and Methods

 Patients

Patients with type 2 diabetes (age, 20 to 75 years) without diabetic complications and insulin therapy were recruited in our hospital to investigate the effects of a single ghrelin administration. Next, patients with type 2 diabetes (age 20 to 75 years) with DPN were
To the patients who were hospitalized, 1.0 μg/kg human ghrelin (100 mL solution) was intravenously administered within 30 min, every morning just after breakfast, for 2 weeks. Body weight, height, and body composition using DXA were measured at baseline and after the 2-week treatment period.

Assessment of DPN
All hospitalized patients were asked to fill a short-form McGill pain questionnaire (SF-MPQ), visual analog scale (VAS) of pain, and the total symptom score (TSS), before and after the 2-week ghrelin administration.

We studied the left posterior tibial nerve for MCV and the left sural nerve for SCV using a Nicolet Viking IV device (Nicolet Biomedical Inc., USA). The nerve conduction velocity tests were performed before and after the 2-week ghrelin administration.

Blood sampling and assay
Liquid meal tolerance tests (400 kcal, carbohydrates 63%; protein 17%; lipids 20%) were done twice in the patients who received a single ghrelin administration. The same liquid meal tolerance tests were done before and after the 2-week ghrelin administration. Blood samples were taken at 0 (baseline), 15, 30, 45, 60, 90, 120, and 180 min postprandial; blood glucose, insulin, C-peptide, GH, ghrelin, and des-acyl ghrelin levels were measured at all blood sampling times. Blood samples were centrifuged and the resulting serum and plasma were stored at –80 °C until assayed. Serum GH concentrations were determined by ELISA (Toso, Tokyo, Japan). C-peptide, 8-isoprostane, 1,5-anhydro-glucitol, glycoalbumin, interleukin-6, and high sensitivity C reactive protein (hs-CRP) levels were measured at SRL Inc. (Tokyo, Japan). For measuring plasma ghrelin and des-acyl ghrelin levels, blood was drawn into chilled tubes containing EDTA-2Na (1 mg/mL) and aprotinin (500 U/mL). Plasma was diluted.

**Table 1** Baseline characteristics of subjects

| Characteristics               | Single administration | 2-week administration | Untreated | 2-week vs untreated p value |
|------------------------------|-----------------------|-----------------------|-----------|-----------------------------|
| Number (men/women)           | 8/3                   | 6/1                   | 3/3       | 0.16                        |
| Age (year)                   | 58.9 ± 3.1            | 59.7 ± 2.8            | 54.3 ± 3.4| 0.28                        |
| Body mass index (kg/m²)      | 27.1 ± 2.4            | 22.7 ± 1.9            | 23.6 ± 1.8| 0.68                        |
| HbA1c (%)                    | 7.3 ± 0.2             | 7.8 ± 0.4             | 9.5 ± 1.3 | 0.28                        |
| Duration of diabetes (year)  | 8.0 ± 1.3             | 15.0 ± 2.9            | 8.6 ± 3.4 | 0.22                        |
with a 10% volume of 1N HCl after centrifugation at 4 °C. Plasma ghrelin and des-acyl ghrelin levels were measured by automated enzyme immunoassay assay (AIA-600II, Tosoh Corporation, Tokyo, Japan).

**Statistical analyses**

All results are presented as means ± standard error. Changes in continuous measures of laboratory test values before and after 2-week ghrelin administration were analyzed using paired t-tests. Non-parametric methods were used for non-normally distributed values. JMP 12.0 (SAS Institute Inc., Cary, NC, USA) were employed for statistical analysis.

**Results**

In the single ghrelin administration group, serum GH levels rapidly increased after ghrelin but not saline administration (data not shown). Both plasma glucose and insulin levels did not change between ghrelin and saline administration (data not shown). These results confirmed the safety of single ghrelin administration in patients with diabetes under these conditions.

Characteristics of seven patients with DPN who were treated with ghrelin and six untreated patients with DPN are shown in Table 1. All ghrelin-treated patients had typical clinical symptoms and signs including numbness, dysesthesia or hyperesthesia of the feet, a decreased or absent Achilles tendon reflex, and shortened vibration perception using a 128-Hz tuning fork (under 10 s). Subjective symptoms of DPN after 2-week ghrelin administration, assessed based on TSS, significantly improved compared to symptoms before treatment, while SF-MPQ and VAS score did not change significantly (Fig. 1A–C). The MCV of the posterior tibial nerve was significantly improved after 2-week ghrelin administration, while MCV did not change in patients without administration of ghrelin (Fig. 1D). The SCV of the sural nerve in three patients could not be detected before ghrelin treatment, but SCV in two of the three patients could be detected after 2-week ghrelin administration. Overall, SCV did not change significantly (Fig. 1E).

Plasma ghrelin concentrations gradually decreased after taking a meal before ghrelin administration; however, it rapidly increased after intravenous ghrelin infusion at 2 weeks after treatment (data not shown). Plasma des-acyl ghrelin concentrations changed in the same way, but the increment was lower than for ghrelin (data not shown). Plasma glucose levels in a liquid meal tolerance test after 2-week ghrelin administration were significantly decreased compared to baseline, while serum C-peptide levels did not change (Fig. 1F–I). Postprandial serum GH concentrations after 2-week ghrelin administration significantly increased compared to those before treatment (Fig. 1J, K). After 2-week administration of ghrelin, glycoalbumin levels significantly decreased and 1,5-anhydroglucitol levels tended to improve, compared to baseline. Plasma and urine 8-isoprostane, interleukin-6, and hs-CRP levels did not change significantly. Although body weight significantly decreased, lean body mass and fat mass did not change after the 2-week ghrelin administration (data not shown).

No noteworthy adverse reactions occurred during this study.

**Discussion**

In the present study, subjective symptoms of DPN assessed based on TSS and MCV in the posterior tibial nerve significantly improved after 2-week ghrelin administration. Although there was no strict control group in this study, MCV in hospitalized patients with DPN who were not treated with ghrelin did not change (Fig. 1D). These results suggest that hospitalization is not essential for improving MCV in DPN.

We tried to determine the underlying mechanisms of the effect of ghrelin in humans, however markers of oxidative stress, inflammation, and interleukin-6 did not change in this study. We previously reported that plasma 8-isoprostane, an oxidative stress marker, decreased significantly after 4-week ghrelin administration to STZ-induced diabetic mice [2]. Ghrelin is reported to have neuroprotective effects. Ghrelin induced significant proliferation of primary cultured cells from the fetal spinal cord [3]. In Parkinson’s disease mice models, ghrelin protected the brain by upregulating UCP-2 and decreasing the production of reactive oxygen species [4]. In Alzheimer’s disease mice models, ghrelin reduced memory deficits, microgliosis, and neuronal loss, and prevented synaptic degeneration [5]. On the other hand, plasma ghrelin levels in patients with DPN were reported to be lower compared to healthy controls [6]. These results suggest that low plasma ghrelin levels may be one of the precipitating causes, and ghrelin replacement may be a novel therapeutic target for DPN.
Fig. 1  The effects of 2-week ghrelin administration to patients with diabetic peripheral neuropathy

Changes are illustrated for (A) subjective symptoms assessed by the total symptom score (TSS), (B) short-form McGill pain questionnaire (SF-MPQ), (C) the visual analogue scale (VAS) of pain, (D) motor nerve conduction velocity (MCV) of the posterior tibial nerve, (E) sensory nerve conduction velocity (SCV) of the sural nerve, (F) plasma glucose levels during a liquid meal tolerance test, (G) glucose AUC 0-180 min, (H) serum C-peptide, (I) C-peptide AUC 0-180 min, (J) growth hormone (GH), and (K) GH AUC 0-180 min, respectively. * p < 0.05 vs. baseline, # p < 0.06 vs. baseline.
Ghrelin was reported to inhibit insulin secretion in humans and rodents. When 1.0 μg/kg of ghrelin was intravenously administered to healthy humans after an overnight fast, serum insulin levels significantly decreased by approximately 2-3 mU/L and plasma glucose levels significantly increased by approximately 5-10 mg/dL [7, 8]. In this study, serum C peptide and plasma glucose levels during a meal tolerance test did not worsen after either single or 2-week ghrelin administration. Diet therapy during hospitalization and postprandial administration of ghrelin might have affected the results in this study, and further investigations are thus needed to clarify the effect of ghrelin on glucose metabolism in patients with diabetes.

In conclusion, our results suggest that neither single nor 2-week administration of ghrelin immediately after taking a meal negatively affect glucose metabolism in patients with type 2 diabetes. Ghrelin may have pleiotropic effects and is expected to be a novel therapeutic target for the treatment of DPN; double-blind, placebo-controlled trials are however needed in the future to investigate the effect of ghrelin on DPN as well as its underlying mechanisms.

Disclosure

None of the authors have any potential conflicts of interest associated with this research.

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