Peritoneal transport characteristics and dwelling time significantly impact ghrelin clearance in peritoneal dialysis patients

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Abstract

Background. Plasma ghrelin exerts widespread bioactivities. Although it is effectively removed from the blood by a single course of haemodialysis, peritoneal clearance of ghrelin is uncertain. Our study aimed to determine (i) whether there is a correlation between plasma ghrelin levels and characteristics of peritoneal ghrelin clearance, and (ii) whether plasma ghrelin levels significantly impact markers of mortality or morbidity in continuous ambulatory peritoneal dialysis (CAPD) patients.

Methods. We enrolled 50 qualified CAPD patients. Blood was drawn during the fasting state and 2 h post-prandially. Also during these periods, peritoneal effluents were collected for radioimmunoassay of total plasma ghrelin level and measurement of other parameters. Twenty-four hour ascites were collected for determination of ghrelin daily mass transfer.

Results. Peritoneal ghrelin clearance was positively correlated with the dialysate-peritoneal creatinine (D/PCr) ratio. Fasting plasma ghrelin levels were inversely correlated with the peritoneal/plasma (D/Pghrelin) ratio (P = 0.045). Plasma ghrelin levels were negatively correlated with body mass index, waist-hip ratio, fasting insulin and triglyceride level, and positively correlated with lean body mass. Plasma ghrelin levels were positively correlated with left ventricular mass (LVM), left ventricular mass index and blood pressure.

Conclusions. Peritoneal transporter characteristics may modulate plasma ghrelin levels in CAPD subjects. By contributing to the level of plasma ghrelin, dwelling time may have an impact on LVM and associated morbidity in CAPD patients.

Keywords: continuous peritoneal ambulatory dialysis; left ventricular mass; plasma ghrelin; visual analogue scales

Introduction

Protein-energy malnutrition is a highly prevalent morbidity in the continuous peritoneal ambulatory dialysis (CAPD) population [1]. Recent studies have shown that the appetite status (measured via visual analogue scale rating) of dialysis patients is a key indicator of their general health and quality of life, and is also a main contributor to nutritional status and clinical outcome [2]. Foley et al. [3] showed that 74% of patients in maintenance dialysis had left ventricular hypertrophy, which was an independent predictor of cardiac events in the Framingham cohort [4]. Cardiac disease is still the leading cause of death among patients receiving long-term dialysis, accounting for 44% of overall mortality [3].

Ghrelin, an endogenous ligand for growth hormone secretagogue receptor, is a 28-amino acid (3300 Da) peptide that is predominantly produced by the stomach, but exerts hormonal actions throughout the body [5]. Besides having orexigenic properties coupled with control of energy homoeostasis, the acylated and desacyl forms of ghrelin exert many significant biological actions [6]. Wynne et al. [7] showed that subcutaneous administration of ghrelin may enhance acute food intake, and result in a significant fall in mean arterial blood pressure in malnourished patients who receive maintenance peritoneal dialysis (PD) [7].

In our previous study in non-diabetic haemodialysis (HD) patients [8], we showed that plasma ghrelin levels are positively correlated with lean body mass (LBM) and left ventricular mass (LVM), and that plasma ghrelin was efficiently cleared during HD. Iglesias et al. [9] demonstrated that PD is accompanied by a striking decrement in baseline ghrelin.
concentration compared with the decline found in HD patients. However, the metabolism of ghrelin during PD is uncertain. The purpose of the present study was to test (i) whether there is a correlation between plasma ghrelin levels and various characteristics of peritoneal ghrelin clearance (ii) whether there is a correlation between characteristics of peritoneal ghrelin clearance and peritoneal creatinine clearance and (iii) whether plasma ghrelin levels significantly impact markers of mortality or morbidity (such as LVM, blood pressure and lipid profile) in CAPD patients.

Subjects and methods

A total of 50 CAPD patients (30 males, mean age 44.26 ± 13.53 years and 20 females, mean age 48.70 ± 12.53 years; NS) were enrolled in the study. Six volunteers that were age- and body mass index (BMI)-matched were recruited for the control group. We excluded patients who had been recently (< 1 month) hospitalized for acute or chronic disorders, who were > 65 years or < 18 years and who had a body weight change >1 kg within 1 month or who were unwilling to participate in the study. The Human Subjects Review Committee at the Chunghua Christian Hospital (CCH)—Taiwan approved all procedures and protocols. A written informed consent was also obtained from all subjects before enrolment.

The plasma from these blood samples and ascites were stored (-70°C) for 24 h to measure daily mass transfer in ghrelin. The efficiency of dialysis was based on the delivery dose of dialysis (Kt/Vurea) using a single-pool urea kinetic model. For an indirect indicator of protein intake, normalized protein nitrogen appearance was calculated from dialysis urea removal and serum urea levels.

The LVM (g), inter-ventricular septum distance (IVSd, mm), left ventricle posterior wall (LVPW, mm), right ventricular diameter (RVD, cm), and ejection fraction (EF, %) were calculated by a cardiologist using a Hewlett-Packard Sonos 2000® (Andover, MA, USA) Phased-Array Imaging System with built-in software from the ultrasonographic unit. These parameters were obtained through a standard procedure of parasternal long-axis view, parasternal short-axis view, apical 4-chamber view and apical 5-chamber view. Left ventricular mass index (LVMi) of these subjects LVM/body surface area, g/m² was calculated from the above data. Wall motion score index (WMSI), a numeric scoring system based on the contractility of less individual segments, was also measured by dividing the sum of wall motion scores by the number of visualized segments to estimate the extent of myocardial ischaemia or infarction. Higher scores (from 1 to 5) indicated more severe wall motion abnormality. All echocardiography measurements were carried out according to the recommendations of the American Society of Echocardiography. Blood pressure was taken twice after a 5-min rest in the echocardiography unit using a random zero sphygmomanometer. Pulse pressure was measured as systolic blood pressure minus diastolic blood pressure.

Plasma immunoreactive ghrelin was measured in duplicate using a radioimmunoassay involving 125I-labelled bioactive ghrelin tracer and a rabbit polyclonal antibody (against full-length, octanoylated human ghrelin) that recognizes the acylated and desacyl forms of the hormone (Phoenix Pharmaceuticals Inc., Belmont, CA, USA). The lower and upper limits of detection were 100 and 1000 pg/ml, respectively. The coefficient of variation was <5% within assays and <14% between assays. We measured haemoglobin using routine procedures with an autoanalyzer from the Department of Clinical Chemistry. Fasting insulin levels (reference range <13 μU/ml) were measured using chemiluminescence immunoassay (Bayer ADVIA Centaur, USA), and homocysteine (reference range <12 μmol/l) was assessed with a microparticle enzyme immunoassay (Abbott Axsym, USA).

Statistical analysis

Ghrelin levels are expressed as mean ± SD. The Spearman correlation coefficient was used to evaluate the linear association between continuous variables. Two-sample Student’s t-tests were performed to compare ghrelin levels between the two groups. ANOVA along with Scheffe’s multiple comparison analysis was used to compare the mass transfer of ghrelin between the high (H), high average (H-A), low average (L-A) and low (L) peritoneal drainage groups. The statistical package SAS v8.02 was used for the analysis, and P-values <0.05 were considered to be of statistical significance.
Results

Peritoneal clearance of ghrelin in CAPD patients

D/P ghrelin values were significantly ($P < 0.01$) greater in 8-h dwelling than in 2-h dwelling periods (Figure 1). In the 8-h dwelling and 2-h dwelling periods, D/P ghrelin levels were significantly ($P < 0.05$) different between peritoneum of high creatinine transport and low-average transport (Figure 1), but the D/P ghrelin level was not different between peritoneum of high creatinine transport and high-average transport or between peritoneum of high-average transport and low-average transport. The ratio of ghrelin concentrations in dialysate and in plasma ranged from $0.51 \pm 0.26$ (low-transport peritoneum) to $0.83 \pm 0.36$ (high-transport peritoneum) in individual collections. Daily ghrelin clearance in dialysate (set of four dialysates from each individual) ranged from $4.9 \times 10^5 \pm 7.7 \times 10^5$ pg (high-transport peritoneum) to $2.6 \times 10^5 \pm 1.8 \times 10^5$ pg (low-transport peritoneum).

Analysis of the effect of peritoneal transport characteristics on ghrelin mass transfer (Figure 2) showed a positive correlation between ghrelin mass transfer amounts in 24 h and the D/P$_{Cr}$ ratio. There was a significant difference between the high (H) transporter and low average (L-A) transporter ($P < 0.05$), but not between the H transporter and H-A transporter or between the H-A and L-A transporters.

Fasting plasma ghrelin levels were inversely correlated with the peritoneal/plasma (D/P$_{ghrelin}$) ratio ($R = -0.25$, $P = 0.045$). However, fasting and post-prandial plasma ghrelin levels were not correlated with weekly creatinine clearance (WCC) or Kt/V (Table 1). In contrast, the D/P ratio (in both 2-H and 8-H) for ghrelin was positively correlated with WCC ($R = 0.44$, $P = 0.002$; $R = 0.43$, $P = 0.003$) and Kt/V ($R = 0.31$, $P = 0.03$; $R = 0.37$, $P = 0.01$). There was an insignificant correlation between glucose load and plasma ghrelin levels ($R = 0.01$, $P > 0.05$) in the CAPD patients.

Correlations between plasma ghrelin levels and body composition, left ventricular mass and lipid profile in CAPD patients

Plasma ghrelin concentrations were positively correlated with LVM ($R = 0.33$, $P < 0.05$) and LVMI ($R = 0.47$, $P < 0.005$) in CAPD subjects (Table 2). There was also a positive but non-significant relationship between plasma ghrelin levels and atrial diameter and EF of the left ventricle in CAPD subjects. Ghrelin clearance via the peritoneum was negatively but insignificantly related to LVM. Both fasting and post-prandial plasma ghrelin levels were positively ($P < 0.05$) correlated with systolic blood pressure; also, post-prandial plasma ghrelin levels were positively ($P < 0.05$) correlated with pulse pressure. In the CAPD subjects with normal levels ($n = 30$) of C-reactive protein (CRP; normal range: $<0.1$ mg/dl), fasting ghrelin levels were $840.63 \pm 330.55$ pg/ml and post-prandial ghrelin levels were $780.64 \pm 370.44$ pg/ml. In patients with higher levels ($n = 20$) of CRP ($> 0.1$ mg/dl), fasting ghrelin levels were $660.13 \pm 180.16$ pg/ml and post-prandial ghrelin levels were $600.65 \pm 180.55$ pg/ml. After adjusting for age and fat mass, fasting and post-prandial levels of plasma ghrelin were lower ($P < 0.01$) in the abnormal CRP group (CRP levels $\geq 0.1$ mg/dl) than in the normal CRP group.
Plasma ghrelin concentrations were negatively correlated with several body fat components (Table 3), including body mass index (BMI), waist-hip ratio (WHR) and triceps skin fold (TSF). Post-prandial plasma ghrelin was positively correlated with LBM, the so-called ‘fat-free component’ in body composition. Both fasting and post-prandial plasma ghrelin levels were negatively correlated with fasting insulin and triglyceride levels, and inversely correlated with cholesterol. Plasma ghrelin level was not correlated with duration of maintenance of peritoneal therapy or EPO® index (EPO dose-haematocrit ratio, EPO/Hct) in our CAPD subjects (data not shown).

Discussion

Leptin is an important 16 KDa endogenous anorexic peptide that counter-regulates the effect of ghrelin, and hyperleptinaemia is commonly observed in patients undergoing chronic dialysis [10]. In contrast to leptin which has little peritoneal clearance [10], the ratio of ghrelin concentrations in dialysate and in plasma ranged from 0.51 ± 0.26 (L-A transporters) to 0.83 ± 0.36 (H transporters). The main determinant of peritoneal clearance for leptin and ghrelin should be the molecular weight. With different peritoneal transporters, D/P ghrelin during 8-h dwelling was significantly greater than during 2-h dwelling. The D/P ratio (in both 2-h and 8-h) for ghrelin was positively correlated with WCC and Kt/V. These findings indicate that ghrelin clearance is more efficient, as is the clearance of small solutes during PD, in the peritoneum with a higher D/PCr or during longer dwelling times. Our study also demonstrated that fasting plasma ghrelin levels were inversely correlated with the peritoneal/plasma (D/P ghrelin) ratio. In agreement with Iglesias et al. [9], we did not find a correlation between glucose load and plasma ghrelin levels. Given the widespread physiological functions of ghrelin, efficient peritoneal ghrelin clearance has important clinical implications and should be further studied.

Recent data showed that ghrelin inhibited apoptosis in cardiomyocytes and endothelial cells through activation of extracellular signal-regulated kinase 1/2 and AKT serine kinase [11]. In a study examining the effects of ghrelin administration on heart failure in humans [12], the authors found that exogenous ghrelin increased both LBM and LVM to thereby increase cardiac output in their heart failure population. Previously, we also found that plasma ghrelin was positively correlated with LVM in HD patients [8]. In the present study (Table 2), we demonstrated that plasma ghrelin concentrations were positively correlated with LVM, LVMI and LBM in CAPD subjects. There was an insignificant correlation between plasma ghrelin levels and bi-atrial/EF of the left ventricle. We also found that endogenous plasma ghrelin was positively correlated with systolic blood pressure in CAPD subjects (Table 2).
Wiley and Davenport [13] demonstrated that ghrelin exerts direct vasorelaxant properties by reversing endothelin-1 induced vasoconstriction [13]. In the current study, our patients with abnormal CRP levels had lower plasma ghrelin concentrations than patients with normal CRP levels. Taken together, these findings suggest that endogenous plasma ghrelin may play a role in regulating cardiovascular system functions.

An emerging role of ghrelin on metabolic syndrome development has been demonstrated in older and type 2 DM subjects [14,15]. In agreement, and in accordance with our findings in HD patients [8], we found that plasma ghrelin levels were negatively correlated with measures of body fat mass (BMI, fat content, WHR and TSF), insulin and TG in CAPD subjects. Ghrelin can regulate energy balance independent of food intake by modulating the metabolic substrate (i.e. fat vs carbohydrate) preferentially used for the maintenance of energy balance [16] and by an inhibition of insulin release [17]. These actions indicate that ghrelin concentrations may have a critical impact on metabolic syndrome development in dialysis patients.

In summary, this study showed that (i) ghrelin is significantly cleared by PD, (ii) peritoneal clearance characteristics and dwelling time plays a role in ghrelin clearance regulation and (iii) plasma ghrelin is significantly correlated with body composition, TG levels and LVM.

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