Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Perturbation of acyl ghrelin profile after liver transplantation

Kohei Murakami, MD, Shuji Takiguchi, MD, PhD, Yasuhiro Miyazaki, MD, PhD, Yukinori Kurokawa, MD, PhD, Makoto Yamasaki, MD, PhD, Hiroaki Nagano, MD, PhD, Masaki Mori, MD, PhD, and Yuichiro Doki, MD, PhD*

Department of Gastroenterological Surgery, Osaka University Graduate School of Medicine, Osaka, Japan

ARTICLE INFO

Article history:
Received 28 January 2015
Received in revised form
13 May 2015
Accepted 12 June 2015
Available online 25 June 2015

Keywords:
Acyl ghrelin
Deacylation
Liver failure
Liver transplantation

ABSTRACT

Background: A significant problem to be solved for patients after liver transplantation (LT) is malnutrition with anorexia in the early posttransplant period. We hypothesized that this problem was due to the change in ghrelin metabolism during LT. The aim of this study was to examine the balance of acyl ghrelin (AG) and desacyl ghrelin and the dependence of the regulation mechanism on hepatic-related enzymes in patients during LT.

Materials and methods: AG, desacyl ghrelin, and acyl/total ghrelin (A/T) concentrations in blood samples were measured in 15 patients with liver failure (LF), 15 patients after LT, and 10 controls. The correlations between the participants' ghrelin profiles and hepatic function–related data, including liver enzymes, were evaluated. *In vitro* assays using synthetic AG for assessment of deacylation activity in serum were performed.

Results: AG and A/T ratio were significantly higher in the LF patients than the patients after LT and controls (AG: 25.9 ± 12.6 *versus* 16.4 ± 12.6 and 9.8 ± 7.6 fmol/mL, $P < 0.05$; A/T ratio: 17.4 ± 4.1 *versus* 12.2 ± 5.5 and 11.8% ± 5.9%, $P < 0.05$). The serum cholinesterase level was inversely correlated with AG and A/T ratio ($P < 0.01$). *In vitro* assays showed that deacylation activity was significantly lower in patients with LF than controls (10.5% *versus* 42.4%, 90 min; $P < 0.01$). Degradation of AG was partially suppressed by a cholinesterase inhibitor.

Conclusions: Deacylation activity was lower in LF patients, which could cause elevation of AG levels. Serum cholinesterase may be responsible for deacylation in humans.

1. Introduction

Ghrelin is a 28-amino acid peptide hormone that was discovered as an endogenous ligand for the growth hormone (GH) secretagogue receptor [1]. Ghrelin has various physiologic functions aside from GH secretion, such as appetite stimulation, improvement of gastrointestinal movement, amelioration of cardiopulmonary functions, and anti-inflammatory effect [2–7]. We previously reported that ghrelin decreased after invasive surgery, such as esophagectomy, and ghrelin administration during the perioperative period was effective for attenuating body weight loss and reducing inflammation in the patients [7–10]. To perform those plentiful biological functions, ghrelin requires an "acylation" process, which corresponds to the attachment of the middle-chain saturated fatty acid to the third serine residue.

* *Corresponding author*. Department of Gastroenterological Surgery, Osaka University Graduate School of Medicine, 2-2-E2, Yamadaoka, Suita, Osaka 565 0871, Japan. Tel.: +81 6 6879 3251; fax: +81 6 6879 3259.
E-mail address: stakiguchi@gesurg.med.osaka-u.ac.jp (S. Takiguchi).

0022-4804/$ – see front matter
http://dx.doi.org/10.1016/j.jss.2015.06.032

Ghrelin is secreted from the X/A-like cell in the fundic glands of the stomach, in which proghrelin is acylated by ghrelin O-acyl transferase, then released to the circulation in its active form [11,12]. However, more than 90% of circulating ghrelin consists of desacyl ghrelin (DG) and less than 10% is acyl ghrelin (AG) [13]. Exogenous acylated ghrelin is rapidly deacylated after intravenous administration [14]. Therefore, deacylation is another important process of ghrelin regulation, although the participating enzymes and organs are not fully elucidated.

The maintenance of hormonal blood levels is one of the key functions of the liver. For example, dysfunction of insulin and GH secretion is frequently reported in patients with liver cirrhosis [15–17]. Whether liver function is involved in ghrelin metabolism remains controversial. It requires several weeks for liver failure patients to improve nutritional status after liver transplantation despite fast recovery of liver function [18]. Perturbation of AG may be related to this malnutrition. The relationship between the circulating enzyme, esterase, to the metabolism of ghrelin, that is, deacylation, has been suggested based on *in vitro* studies [19,20]. Cholinesterase, which is a major component of serum esterase, is synthesized in the liver [21]. Abnormalities in circulating AG levels in patients with liver failure may be attributed to a decline of deacylating enzymes such as cholinesterase.

The aim of this study was to determine the change in AG level after LT, to clarify the deacylation mechanism by examining the correlation between liver function and AG levels, and to evaluate cholinesterase as a deacylation enzyme in human samples. Moreover, to further investigate the deacylation activity of the enzyme, *in vitro* assays using synthetic AG were performed.

2. Materials and methods

2.1. Patients

Three cohorts, including liver failure, post-LT, and control groups were enrolled for this study. The liver failure group consisted of 15 patients who were indicated for LT according to the committee of transplantation at Osaka University Hospital between July 2012 and August 2013. The severity of liver function was assessed according to the Child-Pugh score and the model for end-stage liver disease scores [22]. The causes of liver failure were alcoholism, viral disease, nonalcoholic steatohepatitis, primary biliary cirrhosis, and fulminant hepatitis. The post-LT group included 15 patients who had undergone LT from a living donor between September 2007 and January 2013 and had more than 6 months without significant complications after transplantation. Because five patients of the liver failure group underwent LT during the study periods, these five patients were included in both the liver failure and post-LT groups. As a control (for blood samples of patients with a normal liver), 10 preoperative colorectal cancer patients, who were considered to have no alterations in the gastric production of ghrelin were enrolled [23].

LT was performed according to the well-established clinical protocol at our hospital, and all the patients received a liver transplant from a living donor. Immunosuppression was induced with prednisone and a calcineurin-inhibiting drug.

This study protocol was approved by the Institutional Review Board of the Osaka University Hospital.

2.2. Measurement of ghrelin, GH, insulin-like growth factor 1, and cholinesterase concentrations and other biochemical markers of liver function

Blood samples were collected before breakfast after an overnight fast. Sampled blood was immediately transferred into a chilled glass tube containing disodium ethylenediaminetetraacetic acid for plasma sampling, and samples were then centrifuged at 4°C. Plasma samples were mixed with a 10% volume of 1 N hydrochloric acid (HCl) and stored at −80°C. Serum samples were directly stored after centrifugation at −80°C. Plasma AG and DG were measured using an ELISA assay and sandwich-type enzyme immunoassay kits according to the manufacturer protocols (for ghrelin: Mitsubishi Kagaku Iatron, Inc, Tokyo, Japan) [8]. Total plasma ghrelin (TG) concentrations were calculated as the AG concentration plus the DG concentration. Ratio of AG to TG (A/T ratio) was calculated using the formula (AG × 100/AG + DG). Serum GH levels were measured using Access2 kit, chemiluminescent enzyme immunoassay (Beckman coulter, Inc, Brea, CA). Serum insulin-like growth factor 1 concentrations were measured using the immunoradiometric assay (TFB, Inc, Tokyo, Japan). Serum cholinesterase concentrations were measured using the hexokinase method (SHINO-TEST Corp, Tokyo, Japan). Other laboratory tests were measured at the Laboratory for Clinical Investigation Department at Osaka University Hospital.

2.3. Serial measurement of A/T ratio and cholinesterase before and after liver transplantation

Blood samples of five patients who were followed before and after LT were collected preoperatively and postoperatively as well as at 1, 3, and 6 mo. The A/T ratio and cholinesterase were also measured in each sample following a similar procedure as previously mentioned.

2.4. AG deacylation by serum in patients with liver failure

To investigate the differences of the AG deacylation process in the blood of patients with liver failure and those with normal liver function, the original assay was performed using synthetic human ghrelin. Synthetic human ghrelin, which consists of 99.4% AG and 0.6% DG, was obtained from the Peptide Institute Inc (Osaka, Japan). Synthetic ghrelin (1×10^{-3} μg) was incubated at 37°C for 0, 60, and 90 min with a final volume of 200 μL of human serum of patients with liver failure and with normal liver function. The incubation was stopped by the addition of 1 N HCl (final concentration of 10%) and by setting the samples on ice. After incubation, AG was measured using an ELISA assay. To fix the true concentration of exogenous AG, serum AG concentration of patients, corresponding to endogenous ghrelin, was subtracted from each incubation sample.