

Influences of compounded human ghrelin for pancreatic fistula after distal pancreatectomy in a mouse model

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Ghrelin is a peptide that is secreted from the stomach and plays a role in appetite, weight gain, and skeletal muscle composition. Compounded human ghrelin (CHG) is a candidate drug to improve the nutritional status after pancreatic surgery. However, adverse influences of ghrelin in terms of pancreatic fistula (PF) via the stimulation of exocrine secretion after pancreatectomy are a concern. The present study showed the influences of the administration of CHG with PF after distal pancreatectomy in a mouse model. Distal pancreatectomy was performed on 10-week-old male Wistar rats and 3 µg/kg or 30 µg/kg CHG was injected into the inferior vena cava during laparotomy. Ten rats were divided into two groups: a control group (no injection; n=5) and a CHG group (n=5 for each of 3 µg/kg and 30 µg/kg). Changes of body weight, amount of ascites, and the serum and ascitic amylase and lipase levels were examined on days 1, 3, and 7. In the case of administration of 3 µg/kg ghrelin, changes of body weight, amount of ascites, and serum amylase level were not significantly different between the groups during 7 days. Amylase level in ascites in the ghrelin administration group tended to be lower than that in the control group on day 3 (p=0.083), but there was no difference on days 1 and 7. In the case of a high dose of 30 µg/kg ghrelin, levels of weight loss were not significantly different between the groups. Although the lipase level in the serum and ascites tended to be lower in the ghrelin group than in the control group (p=0.08), the amylase levels in serum and ascites were not significantly different between the groups. There was also no remarkable difference in histological appearance between the groups during 7 days after surgery. The administration of CHG did not induce PF after distal pancreatectomy, which might inhibit lipase secretion in ascites. CHG injection can be safely performed after surgery.

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Introduction

In major pancreatectomy, body weight loss and malnutrition have been reported to be induced due to loss of exocrine and endocrine functions.^{1,2} To induce early recovery after major surgery, nutritional support in the perioperative period should be necessary and hormonal support is one of the most important treatments. However, an effective supportive

treatment has not been established at this stage.

Ghrelin was discovered as an intrinsic ligand of the growth hormone secretagogue receptor (GHS-R) in 1999 by Kojima et al. and Kangawa et al.^{3,4} Endogenous ghrelin was shown to be mainly produced in the stomach. Ghrelin has multiple functions, such as orexigenic action on the hypothalamus or gastrointestinal motility, stimulating growth hormone secretion, anti-inflammatory activities, maintaining the strength

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of skeletal muscle, and various other metabolic functions.³⁻¹¹ In particular, ghrelin is a powerful gastrointestinal hormone that stimulates appetite, which is regulated by the circadian rhythm.¹² Recently, a clinical trial of compounded human ghrelin (CHG) was attempted in order to increase oral feeding and weight gain, inducing early recovery and anti-inflammatory protection after invasive surgeries, namely, gastrectomy and esophagectomy, in cancer patients.¹³⁻¹⁶ In the field of pancreatic surgery, we have also undertaken a clinical trial using CHG in patients who underwent major pancreatectomy (not published yet). However, in such patients, pancreatic fistula (PF) after resection is always a concern, which often induces subsequent severe complications.¹⁷ Ghrelin has activity to stimulate various digestive hormones or peptides, including the stimulation of pancreatic exocrine functions;^{18,19} therefore, adverse effects of PF induced by the administration of ghrelin are a concern after pancreatectomy. However, remarkable complications associated with ghrelin have yet to be fully clarified in a pancreatectomy model. Thus, we have to clarify whether or not CHG stimulates PF after pancreatectomy before its clinical application.

Against the above background, in the present study, we examined the amount of ascetic fluid and the amylase and lipase levels in ascites after distal pancreatectomy in mice, and compared these levels between groups without (the control) and with the administration of CHG in a mouse model *in vivo*.

Materials and methods

Tumor xenograft and CHG administration protocol

Ten-week-old male Wistar rats (body weight 310-435 g; CLEA Japan, Inc., Tokyo, Japan) were used in this study. Under general anesthesia using 90 mg/kg ketamine hydro-

chloride and 10 mg/kg xylazine (JHP Pharmaceuticals LLC, Rochester, MC), a 5 cm laparotomy was performed and the greater omentum was cut. Splenic artery and vein were coagulated and 1.5 cm of distal pancreas from the splenic hilum was cut. A mean of 0.34 g of the pancreas was resected. A total of 3 µg/kg or 30 µg/kg CHG (Peptide Institute, Osaka, Japan) was directly injected into the inferior vena cava during laparotomy. Overall, 10 rats were divided into two groups: a control group (no injection; n=5) and a CHG group (n=5 for each of 3 µg/kg and 30 µg/kg).

In these models, body weight, amount of ascites fluid, and the serum and ascetic amylase or lipase levels were examined on days 1, 3, and 7. Blood was taken from the vena cava under general anesthesia, and heparinized blood was centrifuged at 3000 rpm for 15 minutes to obtain plasma. Amylase and lipase concentrations were measured using an ELISA kit (Wako Pure Chemical, Osaka, Japan).

Statistical analysis

Data are expressed as mean ± standard deviation (SD). Statistical significance was determined by two-way repeated-measure ANOVA, one-way factorial ANOVA, unpaired t-test, and multiple comparison tests by Tukey's method using the statistical package SPSS Statistics 19 (IBM, NY, NY, USA). A P value of less than 0.05 was considered statistically significant.

Results

Administration of ghrelin at 3 µg/kg

Figure 1 shows the changes of body weight after surgery for the control and ghrelin administration groups; there were

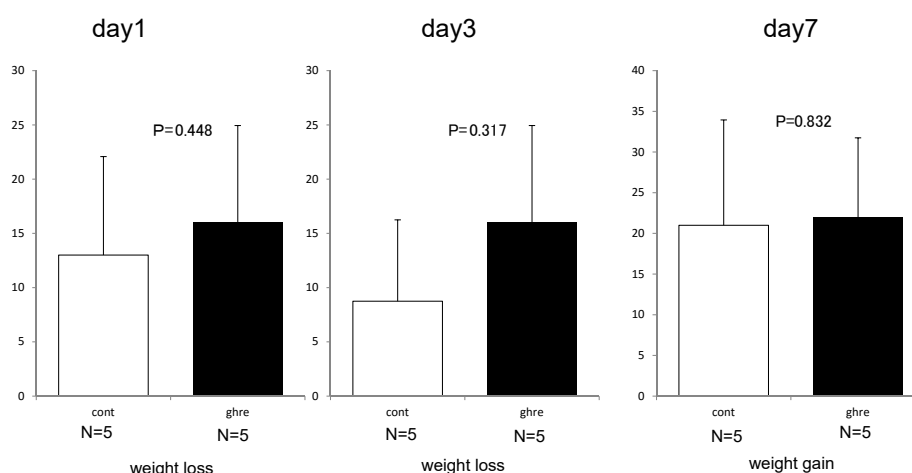


Figure 1. The *in vivo* model. Changes of weight after surgery (3 µg/kg ghrelin).

no significant differences between the groups on days 1, 3, and 7. Figure 2 shows the changes of the amount of ascites after surgery; there were no differences in this variable between the groups during 7 days. Figure 3 shows the changes of serum amylase level after surgery; there were also no differences in the changes of this variable between the groups

during 7 days. Figure 4 shows the changes of amylase level in ascites after surgery; there were no significant differences in this variable between the groups on days 1 and 7. However, amylase level in the ghrelin administration group tended to be lower than that in the control group on day 3 ($p=0.083$).

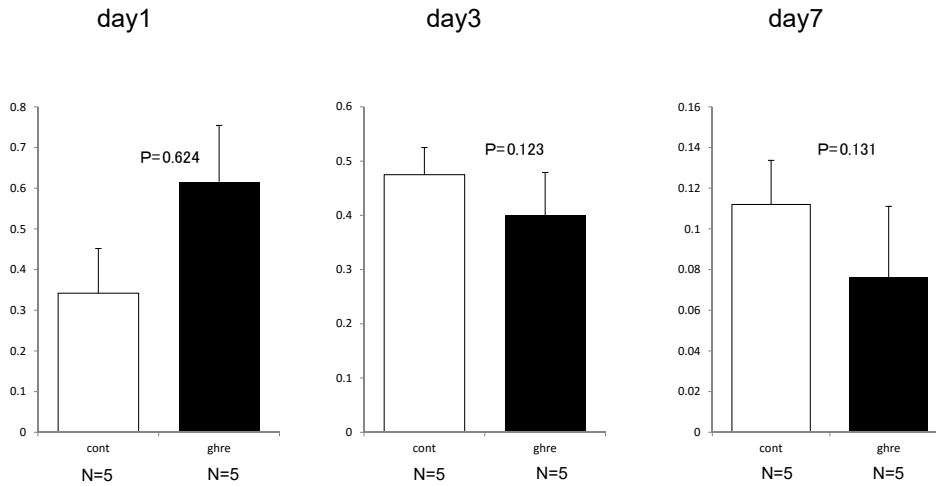


Figure 2. Changes of amount of ascites after surgery (3 $\mu\text{g/kg}$ ghrelin).

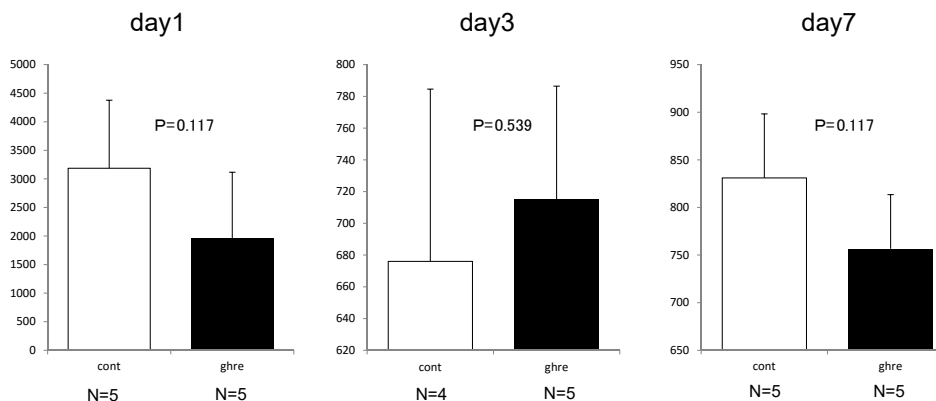


Figure 3. Changes of serum amylase level after surgery (3 $\mu\text{g/kg}$ ghrelin).

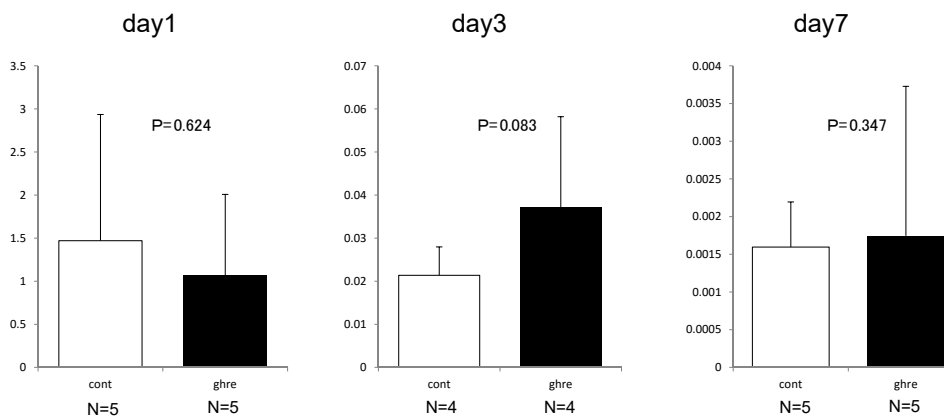


Figure 4. Changes of ascites amylase level after surgery (3 $\mu\text{g/kg}$ ghrelin).

Administration of ghrelin at 30 $\mu\text{g}/\text{kg}$

Figure 5 shows the changes in terms of weight loss at day 1 after surgery; there were no significant differences in this variable between the groups. Figure 6 shows the enzyme levels on day 1 after surgery. Although the amylase levels in serum and ascites were not significantly different between the groups, the lipase levels in serum and ascites tended to be lower in the ghrelin group than in the control group, but the differences were not statistically significant ($p < 0.1$), as

shown in Figure 7. On day 7, levels of weight gain and enzyme levels in the serum and ascites were not significantly different between the groups, as shown in Figure 8.

Pathological findings after ghrelin administration

Figure 9 shows the histological findings of the remnant pancreas. There were no marked differences in terms of the histological appearance between the groups during the 7 days after surgery.

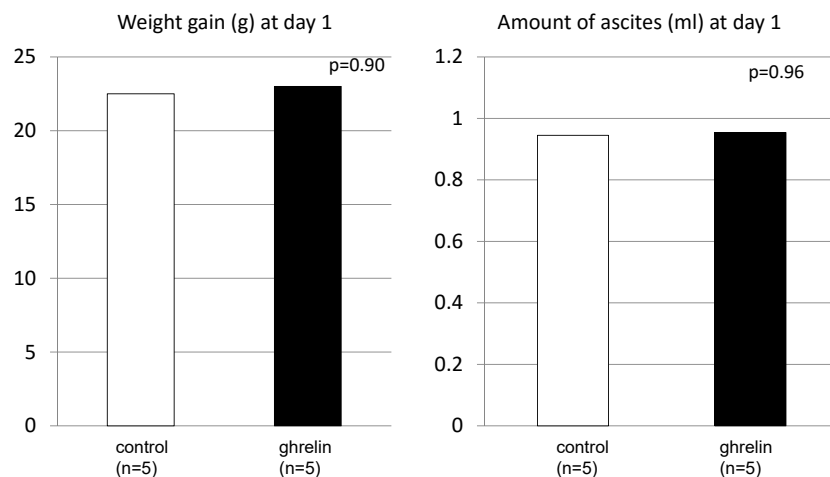


Figure 5. Changes of weight after surgery (30 $\mu\text{g}/\text{kg}$ ghrelin).

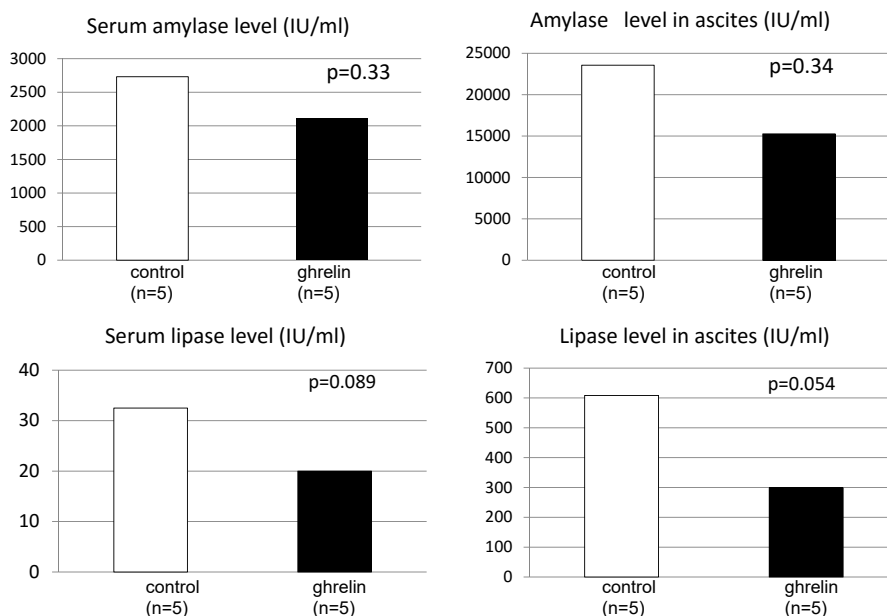


Figure 6. Changes of enzyme level on day 1 after surgery (30 $\mu\text{g}/\text{kg}$ ghrelin).

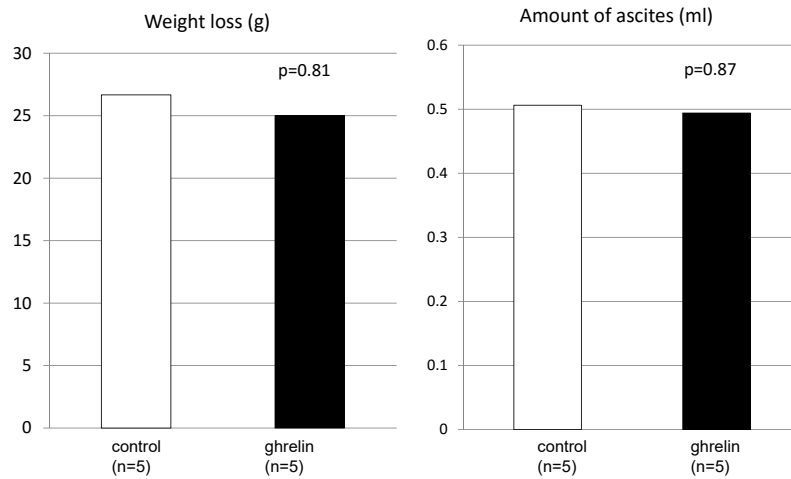


Figure 7. Changes of weight on day 7 after surgery (30 µg/kg ghrelin).

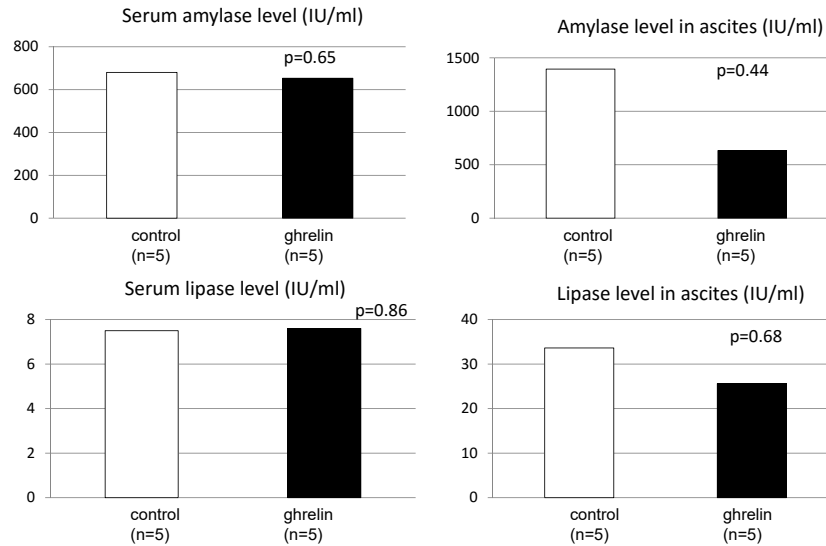


Figure 8. Changes of enzyme level on day 7 after surgery (30 µg/kg ghrelin).

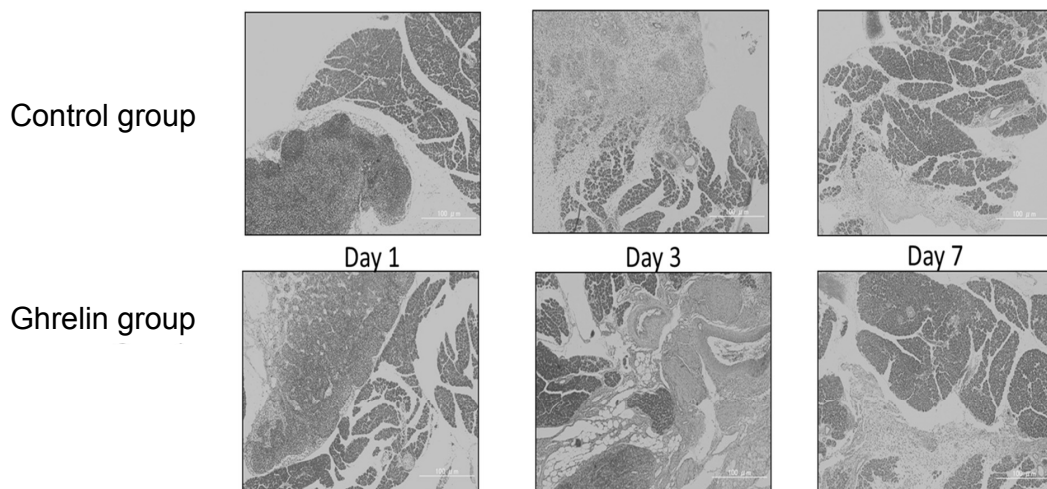


Figure 9. Pathological appearance after surgery (30 µg/kg ghrelin). On day 1, inflammatory cells such as lymphocytes and fibroblasts accumulated in the pancreas stump. On day 3, mild accumulation of inflammatory cells was observed and, on day 7, weak accumulation of inflammatory cells was observed. There were no marked differences between the control and ghrelin groups.

Discussion

Ghrelin induces pancreatic endocrine and exocrine secretion via the brain-gut axis system,²⁰⁻²² and pancreatic cellular proliferation as well as growth hormone.^{3-11, 22} Thus, stimulation of pancreatic fistula by ghrelin administration is a concern clinically. In the present study, we examined such adverse effects of CHG on remnant pancreas before applying ghrelin to enhance patients' nutritional recovery after pancreatic resection. If the pancreatic juice or amylase level in serum or ascites increases, the induction of PF by ghrelin administration is a real concern. Thus, in the present study, a distal pancreatectomy animal model was established and ghrelin was directly injected into the circulation.

Previous reports have described that exogenous ghrelin is an important modulator of endocrine and exocrine functions, or the proliferation and regeneration of islet cells.^{23, 24} Blood glucose level was decreased by ghrelin administration. Ghrelin might thus provide a therapeutic approach to regulate diabetes.^{18, 25, 26} Sato et al. reported that ghrelin stimulates pancreatic exocrine secretion,²⁷ but on the other hand, Kapić et al. reported that ghrelin inhibits the secretion of pancreatic juice in rats.²⁸ The influences of ghrelin administration after pancreatectomy thus remain unclear.

We firstly attempted to administer 3 µg/kg ghrelin in the present study. Changes of body weight, amount of ascites, and serum and ascites amylase levels were not significantly different between the control and ghrelin groups. We thought that a concentration of 3 µg/ml for human administration might be low for the animal model. Indeed, from our results, the administration of this low concentration of ghrelin did not influence pancreatic function or surgical outcome. As a next step, a higher concentration of ghrelin of 30 µg/kg was administered. Although body weight and amylase levels in serum and ascites were not significantly different between the groups, lipase levels in serum and ascites in the ghrelin group tended to be lower than in the control group on day 1. However, this difference was not observed on day 7. A previous report showed that a higher amylase level in ascites could be used to predict PF clinically²⁹; therefore, control of exocrine secretion at an early stage after pancreatectomy is necessary to prevent subsequent clinical PF. Our results might indicate the effect of ghrelin administration on inhibiting pancreatic juice secretion. Histological inflammatory changes during 7 days after distal pancreatectomy did not differ between the groups. Ghrelin administration might not influence the histological architecture or inflammatory responses, although Pantic et al. reported that ghrelin affected the structural complexity of the architecture of exocrine pan-

creatic tissue.²⁴

In the future, drugs of ghrelin or its agonist will be applied for appetite enhancement and to improve anabolic activity.³⁰ Doki et al. reported the usefulness of ghrelin administration during the peri-operative period in surgery of the stomach and esophagus, which improved appetite, food intake, and the nutritional status, as well as having an anti-inflammatory effect.¹⁴⁻¹⁶ In the field of pancreatic surgery, if the problem of adverse effects is resolved, ghrelin would be a promising drug to improve postoperative nutritional status, strengthen skeletal muscle, and relieve severe inflammatory responses.

In conclusion, adverse effects of ghrelin injection at a low concentration were not observed after distal pancreatectomy in an animal model, and lipase levels in serum and ascites in the early period were reduced by the administration of a high concentration of ghrelin, but no adverse effects of the ghrelin were observed *in vivo*. Ghrelin applied in treatment for nutritional improvement in patients with malnutrition is considered not to promote pancreatic fistula in the case of pancreatectomy.

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