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ORIGINAL ARTICLE

Postoperative decrease in plasma acyl ghrelin levels after pediatric living donor liver transplantation in association with hepatic damage due to ischemia and reperfusion injury

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Abstract

Purpose Ghrelin was recently reported to promote recovery from hepatic injury. We hypothesized that it could also be associated with clinical recovery of the transplanted liver from ischemia and reperfusion injury. Our aims were to investigate perioperative ghrelin changes following pediatric living donor liver transplantation (LDLT) and to analyze the association of these changes with postoperative hepatic function.

Methods We measured plasma acyl ghrelin (AG) concentrations before surgery, at the end of surgery and on postoperative days (PODs) 1, 3 and 7 in 12 children who underwent LDLTs, and, as controls, pre- and post-operatively and on POD1 in 7 children who underwent benign abdominal mass resection. The correlations between the participants' ghrelin profiles and hepatic function-related data were evaluated.

Results AG levels significantly declined to 15.6% of preoperative levels after LDLT and almost returned to baseline on POD3. Post-operative AG levels were significantly reduced to a greater extent following LDLT than benign abdominal mass resection. AG levels on POD1 inversely correlated with aspartate aminotransferase levels and cold/total ischemia time ($P < 0.05$).

Conclusion These results suggest that reduced AG levels on POD1 may reflect the degree of damage to the transplanted liver due to ischemia and reperfusion injury.

Keywords Ghrelin · Liver transplantation · Children

Introduction

In 1999, ghrelin was identified as a novel peptide hormone secreted mainly in the fundic glands of the stomach and was found to be an endogenous ligand for the growth hormone secretagogue receptor [1]. Given the variety of its biological functions, including stimulation of appetite and gastrointestinal activity, improvement of cardiopulmonary function, and suppression of excessive inflammatory responses [2–5], clinical studies on ghrelin have extended to some adult diseases, such as gastric and esophageal cancer with subsequent surgery [6, 7]. However, few studies have reported ghrelin levels in pediatric patients and, moreover, to the best of our knowledge, no studies have investigated perioperative ghrelin levels in the field of pediatric surgery.

Recently, ghrelin has been reported to exert protective effects following hepatic injury [8–11]. We hypothesized that these effects could be clinically associated with recovery of the transplanted liver from ischemia and reperfusion injury after living donor liver transplantation (LDLT). Our aims were to investigate perioperative ghrelin changes in pediatric LDLT patients, and to analyze the association between these changes and perioperative clinical data related to hepatic function.

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Methods

Study population and protocol

Twelve patients who underwent LDLT after the Kasai procedure for biliary atresia from January 2015 to December 2017 were enrolled in this study. LDLT was performed according to the well-established clinical protocol at our hospital. All the donors were in healthy condition with normal liver function. Immunosuppression was induced with prednisone and a calcineurin-inhibiting drug. Seven patients with normal liver function who underwent laparotomy for resection of benign abdominal masses, including three cases with mature ovarian teratomas, two with abdominal lymphangiomas and two with urachal remnants, were also enrolled in this study as controls. Written informed consent was obtained from all the patients' parents or guardians. A registration center at the Department of Pediatric Surgery, Osaka University Graduate School of Medicine, was responsible for the registration and prospective monitoring of subjects. This prospective cohort study was approved by our institutional review board (IRB number: 13439).

Study protocol

In LDLT patients, fasting blood samples were obtained five times during the perioperative period to measure serial perioperative changes in plasma ghrelin concentrations: before surgery (Pre-Op), at the end of surgery (Post-Op), and on postoperative days (PODs) 1, 3 and 7; blood samples in controls were obtained three times: Pre-Op, Post-Op and on POD1. The primary endpoint of this study was to evaluate serial perioperative changes in plasma ghrelin concentrations in pediatric LDLT patients in comparison with controls. The secondary endpoints evaluated were the associations between these changes and postoperative hepatic function, as indicated by plasma levels of total bilirubin, hepatobiliary enzymes, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), hepatic reserve, as indicated by prothrombin time, albumin and cholinesterase, and perioperative factors related to liver function, including operative time, operative blood loss and cold/warm/total ischemia time.

Measurement of plasma ghrelin levels and hepatic function-related data

Three millilitre blood samples were obtained and immediately transferred into chilled glass tubes with aprotinin/ethylenediaminetetraacetic acid (EDTA) solution. After centrifugation at 4 °C, plasma was separated and mixed with a 10% volume of 1 N hydrochloric acid (HCl) and stored at − 30 °C. Ghrelin consists of two major molecular forms, acyl ghrelin (AG), which is recognized as an active form of ghrelin, and des-acyl ghrelin (DG) [12, 13]. More than 90% of circulating ghrelin is in the form of DG and less than 10% is as AG [14]. Additionally, rapid deacylation of AG is observed after intravenous administration of exogenous AG [15]. Therefore, deacylation is an important factor in the metabolism of ghrelin. Based on these findings, we considered it necessary to measure not only AG levels, but also the ratio of AG to DG (AG/DG ratio) to evaluate the influence of deacylation. Additionally, evaluation of the AG/DG ratio can be used to exclude intraoperative hemodilution as a possible cause of the reduction in AG levels. Plasma AG and DG levels were measured using a fluorescence enzyme immunoassay (FEIA; Tosoh Corp., Tokyo, Japan), as we previously reported [16]. The AG/DG ratio was calculated using the formula (AG/DG × 100). Other laboratory tests were conducted at the Laboratory for Clinical Investigation at Osaka University Hospital.

Statistical analysis

Statistical differences in plasma AG levels and the AG/DG ratio at various time points in the clinical course were calculated by the Wilcoxon test, paired *t* test or Friedman test. Statistical differences between LDLT and control groups were calculated by the Mann–Whitney *U* test, Welch's *t* test or Fisher's exact test. The relationship between parameters was investigated by Spearman correlation analysis. Statistical significance was set at $P < 0.05$. All calculations were performed using GraphPad Prism 6 software (GraphPad Software Inc., San Diego, CA, USA).

Results

Patient characteristics and perioperative outcomes

No significant differences in sex were observed between the LDLT and control groups. Age in the LDLT group was significantly lower than that in the control group. All the preoperative liver function data, such as serum concentrations of AST, ALT, prothrombin time, total bilirubin and albumin, indicated significantly poorer liver function in the LDLT group than in the control group ($P < 0.01$). In the LDLT group, the median (range) operation time was 681.5 (532–1005) min and median blood loss was 50.6 (20.2–292) mL/kg body weight. Median (range) cold, warm and total ischemia times were 87.5 (71–229), 38 (28–47) and 122.5 (110–274) minutes, respectively. There were no intraoperative complications in both groups. On POD7, which was the last point of blood sample collection for plasma ghrelin

measurement, acute cellular rejection was observed in two cases.

Changes in plasma AG levels following LDLT in comparison with abdominal mass resection

As shown in Fig. 1, in LDLT patients, mean plasma AG levels before surgery, at the end of surgery, and on PODs 1, 3 and 7 were 38.4 ± 9.5, 6.0 ± 2.0, 16.3 ± 4.7, 40.1 ± 13.5, and 43.4 ± 6.9 fmol/mL, respectively. Preoperative AG levels did not differ significantly between LDLT (median 22.2; range 5.9–99.5 fmol/mL) and control groups (median 18.7; range 0.4–55.0 fmol/mL). As shown in Table 1, the percentage ghrelin concentrations represent the percentage of plasma AG levels at the end of surgery and on POD1 relative to its concentration before surgery. The values were significantly lower in the LDLT group than in the control group both at the end of surgery and on POD 1.

Changes in AG/DG ratio following LDLT in comparison with abdominal mass resection

As shown in Fig. 2, in LDLT patients, the mean AG/DG ratios and their standard errors measured before surgery, at the end of surgery, and on PODs 1, 3 and 7 were 62.7 ± 14.1, 15.1 ± 4.6, 79.3 ± 34.3, 210.1 ± 95.6, and 95.2 ± 16.1%, respectively. The results indicate that by the end of LDLT, the AG/DG ratio declined significantly to 24.2% of the preoperative level, almost returned to baseline on POD1, and exceeded the preoperative level on POD3 (significantly increased to 335% of the preoperative level). As shown in Table 1, preoperative AG/DG ratios did not differ significantly between the LDLT (median 41.2; range 10.9–160.5%) and control groups (median 59.6; range 2.1–121.1%). The AG/DG ratios were significantly lower in the LDLT group than the control group at the end of surgery, although they

Table 1 Comparison of plasma acyl ghrelin levels and the ratio of acyl ghrelin to des-acyl ghrelin between LDLT and abdominal mass resection patients

	LDLT patients ($n=12$)	Abdominal mass resection patients ($n=7$)	P value
Plasma AG levels (% of Pre-Op)			
Post-Op	12.2 (0–48.9)	44.6 (29.9–1811)	**< 0.01**
POD1	40.8 (19.4–218.5)	84.1 (39.7–2603)	**< 0.05**
AG/DG ratio (%)			
Pre-Op	41.2 (10.9–160.3)	59.6 (2.1–121.1)	0.90
Post-Op	10.1 (0–57.6)	40.4 (7.7–49.1)	**< 0.05**
POD1	35.7 (3.6–432.4)	35.6 (10.7–114.1)	0.77

Data are expressed as median (range)

The bold font used for certain values indicates significant P values

P values were derived using Welch's t test or Mann–Whitney U test

LDLT living donor liver transplantation, *AG* acyl ghrelin, *DG* des-acyl ghrelin, *Pre-Op* before surgery, *Post-Op* at the end of surgery, *POD* postoperative day

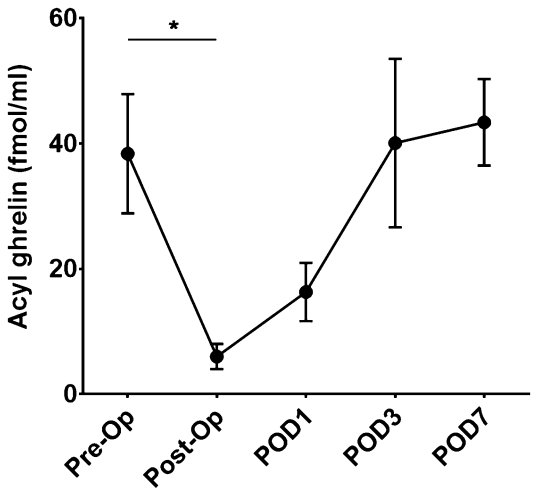

Fig. 1 Serial changes in mean plasma acyl ghrelin levels after living donor liver transplantation. *Pre-Op* before surgery, *Post-Op* at the end of surgery, *POD* postoperative days. Data of the 12 LDLT patients are expressed as means with standard errors. *$P<0.05$, significant decrease in acyl ghrelin levels after LDLT, as assessed by the Wilcoxon test

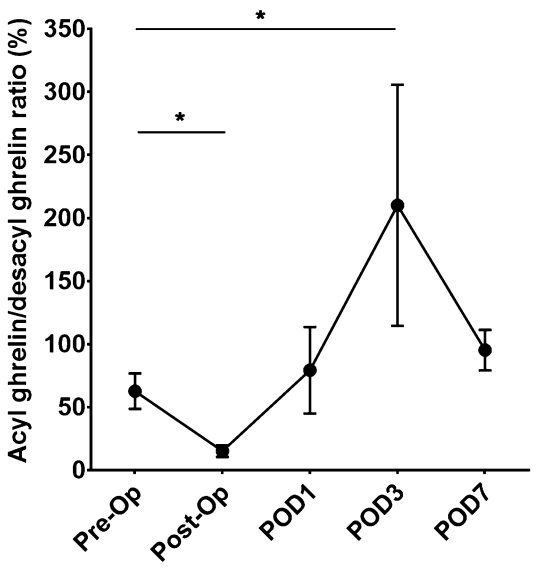

Fig. 2 Serial changes in mean ratio of plasma acyl ghrelin to des-acyl ghrelin after living donor liver transplantation. *Pre-Op* before surgery, *Post-Op* at the end of surgery, *POD* postoperative days. Data of the 12 LDLT patients are expressed as means with standard errors. *$P<0.05$, ratio of plasma acyl ghrelin to des-acyl ghrelin significantly differed as compared to that before surgery, as assessed by the Friedman test

were not significantly different between the two groups on POD 1.

Plasma AG levels on POD1 inversely correlated with AST levels and cold/total ischemia time

Recovery from deterioration of AG levels started on POD1. Therefore, to focus on the AG levels on POD1, the relationship between AG levels and laboratory data related to liver function on POD1 was investigated, as shown in Table 2. Plasma AG levels inversely correlated with AST levels ($r = -0.699$, $P < 0.05$), but not with the other laboratory data, including ALT, prothrombin time, albumin, cholinesterase and total bilirubin. Plasma AG levels also inversely correlated with cold/total ischemia time ($r = -0.657$, -0.590, respectively, $P < 0.05$), but not with operative time, operative blood loss and warm ischemia time.

Discussion

This study is the first to focus on perioperative ghrelin changes in pediatric patients undergoing liver transplant surgery. In the present study, two new findings were reported. First, plasma AG levels and AG/DG ratio are significantly reduced after LDLT in pediatric patients. Second, plasma AG levels inversely correlate with AST levels on POD1, and with cold and total ischemia time, but not with operative time and operative blood loss. These findings suggest that the reduction in AG levels might be related to ischemic injury to the transplanted liver.

Table 2 Correlation between acyl ghrelin levels and clinical data related to liver function measured on postoperative day 1 in LDLT

Correlation analysis compared to acyl ghrelin levels	*r*	*P* value
Laboratory data related to liver function		
AST (IU/L)	− 0.699	**< 0.05**
ALT (IU/L)	− 0.161	0.62
PT (%)	0.217	0.49
Alb (g/dL)	− 0.095	0.77
Cholinesterase (IU/L)	0.084	0.80
T-Bil (mg/dL)	− 0.032	0.93
Perioperative outcomes related to liver function		
Operative time (min)	− 0.196	0.54
Operative blood loss (mL/kg body weight)	0.126	0.70
Cold ischemia time (min)	− 0.657	**< 0.05**
Warm ischemia time (min)	0.092	0.78
Total ischemia time (min)	− 0.590	**< 0.05**

The correlation was analyzed by Spearman's test

The bold font used for certain values indicates significant *P* values

LDLT living donor liver transplantation, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *PT* prothrombin time, *Alb* albumin, *T-Bil* total bilirubin

The protective effects of ghrelin on hepatic injury have been confirmed in several basic research studies using mice models of hepatic injury due to biliary obstruction, acetaminophen, ischemia and reperfusion or lipopolysaccharides [8–11]. Qin et al. described that ghrelin had protective effects on hepatic injury induced by ischemia and reperfusion. In their study using ghrelin receptor gene knockout mice, they demonstrated that not only exogenous administration of ghrelin, but also endogenous ghrelin contributed to protection of hepatic tissue [10]. These findings suggest that the reduction of AG levels after LDLT seen in this study was caused by the consumption of endogenous AG during recovery of the transplanted liver from hepatic injury.

Ischemia and reperfusion injury remains a major problem in clinical transplantation, representing more than 10% of early transplant failures and leading to a higher incidence of both acute and chronic rejection [17–19]. Therefore, therapies that reduce ischemia and reperfusion injury might improve mortality and prevent several of the complications of liver transplantation. Several biological functions of ghrelin, including cytoprotective effects on the liver and suppression of inflammation [5, 8–11], might contribute to the treatment of ischemia and reperfusion injury. Qin et al. also reported that pretreatment with ghrelin significantly attenuated elevations in serum hepatic enzyme levels and tissue damage induced by hepatic ischemia and reperfusion injury in mice [10]. Since endogenous AG greatly declines after pediatric LDLT, perioperative administration of AG to pediatric LDLT patients might be clinically useful in preventing damage to the transplanted liver due to ischemia and reperfusion injury. Since ischemia and reperfusion injury is not specific to LDLT, our results can also probably be applied to DDLT.

Prednisolone was used intra- and postoperatively in the LDLT patients in this study. A previous report demonstrated that hydrocortisone administration was positively associated with plasma AG levels in healthy adults [20]. Therefore, the administration of prednisolone during and after LDLT might not affect postoperative reduction of plasma AG levels. In fact, greater postoperative reduction in plasma AG levels might have been observed if prednisolone was not administered. It is also possible that early postoperative recovery of plasma AG levels or the significant increases in AG/DG ratios on POD3 might have been caused by its administration.

In the field of adult surgery, decline in ghrelin levels after esophagectomy and gastrectomy have been reported [6, 7]. The mechanism of ghrelin decline after gastrectomy might be a decrease in ghrelin production; however, the mechanism after esophagectomy is reportedly unknown [6]. From our results, endogenous AG reduction in LDLT patients might

be a result of consumption of AG by the transplanted liver for recovery from damage. However, a lesser decline in AG levels was observed after abdominal mass resection. These results suggest that postoperative reduction in plasma AG levels might be caused by several factors, among which damage to the transplanted liver might be one factor. Although lack of statistical correlation between plasma AG levels and both operative time and operative blood loss in LDLT indicates that postoperative AG reduction might not be associated with surgical invasiveness, further study is required to investigate the mechanism of AG decline after surgery.

In the present study, plasma AG levels on POD3 in the LDLT patients were almost the same as preoperative levels, while the AG/DG ratio was significantly higher than that before surgery. In fact, total ghrelin levels on POD3 did not completely recover to preoperative total ghrelin levels (data not shown). These findings suggest that plasma AG levels were likely preserved by deacylation of AG. Murakami et al. described that deacylation of AG is inhibited in the presence of liver failure [21]. Therefore, plasma AG levels on POD3 might have been maintained at preoperative levels by deacylation of AG due to postoperative low-grade functioning of the transplanted liver.

One of the limitations of this study is the small number of cases. Since this study was not completely non-invasive, because three mL of blood was obtained at every time point in the study protocol, which is invasive to patients, particularly to infants, we kept the number of subjects to as few as possible. Although a significant result was obtained, more meaningful results could be obtained with a larger number of cases. Another limitation is that the control group was not optimal. Abdominal mass resection is less invasive compared to LDLT. Additionally, patient age at surgery was significantly different between the two groups. As LDLT is one of the most invasive surgeries in pediatric patients, it is difficult to set an appropriate control with similar invasiveness. Therefore, we used patients who underwent laparotomy for abdominal mass resection as controls. Importantly, the strength of our study is that it reveals perioperative changes in ghrelin levels in pediatric LDLT patients. Additionally, we demonstrated a significant correlation between postoperative reduction in plasma AG levels and damage to the transplanted liver only among the LDLT cases.

In conclusion, this report is the first to document perioperative changes in plasma AG levels in pediatric LDLT patients, and to show that the reduction in AG levels might directly correlate with the rise in serum AST levels and cold/total ischemia times. These results suggest that postoperative reduction in AG levels might reflect the degree of damage to the transplanted liver secondary to ischemia and reperfusion injury. Additionally, our results suggest that perioperative ghrelin administration might be a potential therapeutic strategy for recovery from hepatic injury during or after LDLT. The present study, in conjunction with previous reports, supports the need for future studies to investigate the clinical efficacy of administration of ghrelin to pediatric LDLT patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest associated with this manuscript.

Ethical standards This study was approved by the Ethics Committees of Osaka University Hospital (IRB number: 13439).

Informed consent Written informed consent was obtained from the parents or guardians of all patients.

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