

Original Article

Portal modulation effects of terlipressin on liver regeneration and survival in a porcine model subjected to 90% hepatectomy

Hye-Sung Jo¹, Hyun-Jin Park², Yoon Young Choi², Jin-I Seok², Jae-Hyun Han³, Young-In Yoon⁴, Dong-Sik Kim¹

¹Division of HBP Surgery and Liver Transplantation, Department of Surgery, Korea University College of Medicine, Seoul, Korea; ²Department of Biomedical Science, Korea University College of Medicine Graduate School, Seoul, Korea; ³Department of Surgery, College of Medicine, St. Vincent's Hospital, The Catholic University of Korea, Seoul, Korea; ⁴Division of Hepatobiliary Surgery and Liver Transplantation, Department of Surgery, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

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Abstract: Background: Excessive postoperative portal pressure is associated with post-hepatectomy liver failure and small-for-size syndrome after partial liver transplantation. This study aimed to identify the portal modulation effects of terlipressin on liver regeneration and survival in a porcine model subjected to 90% hepatectomy. Methods: Twenty pigs undergoing 90% hepatectomy were divided into control (n = 10) and terlipressin (n = 10) groups. Terlipressin 0.5 mg was injected subcutaneously three times a day, from immediately before hepatectomy to 7 days after surgery, for surviving pigs in the terlipressin group. Portal pressure measurement, biochemical analysis, assessment of molecular markers for liver regeneration, and immunohistochemistry were performed in both groups. Results: The 7-day survival rate was significantly higher in the terlipressin group than that in the control group. Portal pressure in the terlipressin group was lower than that in the control group at 30 min and 1 h after hepatectomy. Total bilirubin level was lower in the terlipressin group than that in the control group at 1 h and 6 h after hepatectomy. Proliferating cell nuclear antigen expression was higher in the control group than that in the terlipressin group at 6 h after hepatectomy, while the proportion of Ki-67-positive cells was higher in the terlipressin group than that in the control group at 7 days after hepatectomy. Endothelin-1 level reflecting liver injury was lower in the terlipressin group than that in the control group at 1 h and 6 h after hepatectomy. Conclusion: Terlipressin could optimize liver regeneration and improve survival through rapid and effective portal modulation after extensive hepatectomy.

Keywords: Terlipressin, portal modulation, liver generation, hepatectomy

Introduction

Liver resection and transplantation are the primary strategies of curative treatment for various hepatic tumors and end-stage liver disease [1]. These surgical interventions are the best treatment options considering the long-term outcomes but could cause severe and fatal complications. Post-hepatectomy liver failure (PHLF) occurs when the small remnant liver fails to meet metabolic demands after extensive hepatectomy [2]. Small-for-size syndrome (SFSS) occurs when hepatic dysfunction develops without a specific cause after partial liver transplantation using a small-for-size graft [3]. In the early postoperative period, increased portal pressure functions as an initial signal for

liver regeneration [4]. However, excessively high portal pressure may cause the small remnant liver or graft to experience shear stress, resulting in sinusoidal endothelial injury with microcirculatory impairment and irreversible liver failure due to the disturbance of effective liver regeneration [5, 6]. Therefore, excessively high portal pressure is regarded as a common pathophysiology in the development of PHLF and SFSS [7, 8]. Several invasive procedures, including splenic artery ligation, splenectomy, and hemiportocaval shunt, have been used to modulate increased portal pressure after partial liver transplantation [9, 10]. However, such procedures increase the risk of severe complications and cannot be adjusted in situations where the portal pressure needs to be modified [11]. Al-

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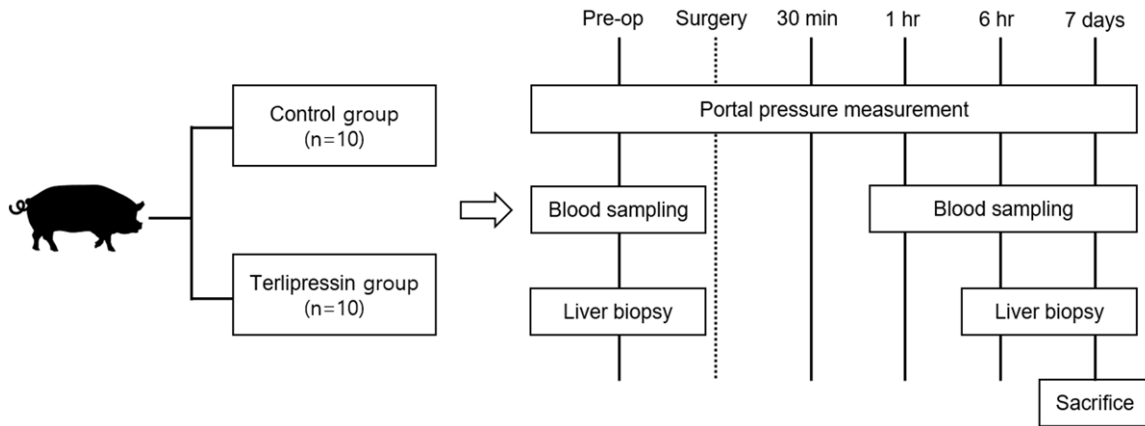


Figure 1. Schematic diagram of the experimental design. A total of 20 pigs were divided into two groups: control (n = 10), terlipressin (n = 10). Portal pressure measurement, blood sampling, and liver biopsy were performed according to the scheduled time frame.

ternatively, some studies have focused on pharmacologic portal modulation using splanchnic vasoactive agents [12]. A previous study in which a porcine model was subjected to 70% hepatectomy reported that terlipressin and octreotide effectively reduced excessive portal pressure and attenuated liver injury after massive hepatectomy [13]. However, to validate the effectiveness of pharmacologic portal modulation for the treatment or prevention of aggressive PHLF or SFSS in clinical settings, its effects have to be proven in a more extensive hepatectomy animal model.

Therefore, in the present study, we aimed to elucidate the mechanism underlying the portal modulation effects of terlipressin on liver regeneration and survival using a porcine model subjected to 90% hepatectomy.

Materials and methods

Study design

This study was approved by the Korea University Institutional Animal Care and Use Committee (KOREA-2016-0129-C1) and strictly followed the guideline for the “Animal Research: Reporting in Vivo Experiments” [14]. The study included 65 to 85 days-old female domestic pigs [median weight 34.9 (range 28.0-39.4) kg]. Pigs were housed in a room with regulated temperature and humidity. All pigs were exposed to a 12 h light/dark cycle. Pigs fasted for 8 h prior to surgery, and all procedures were conducted under sterile conditions.

A total of 20 pigs were divided into the control (n = 10) and terlipressin (n = 10) groups. As previously described [13], the initial dose of terlipressin was determined based on that used for variceal bleeding or hepatorenal syndrome treatments in clinical settings. However, the dose was reduced due to side effects such as peripheral cyanosis or sustained tachycardia in initial cases. Therefore, 0.5 mg terlipressin (Glypressin®, Ferring, Switzerland) was injected intramuscularly three times a day just before hepatectomy to 7 days after surgery for surviving pigs. Blood samples and liver tissues were obtained, and portal pressure was measured according to the scheduled time frame (**Figure 1**). All pigs were followed up until 7 days after surgery, at which time the surviving pigs were sacrificed.

Surgical procedure for 90% hepatectomy in the porcine model

General anesthesia and 70% hepatectomy were performed as previously described [13]. In addition to 70% hepatectomy, half of the right lateral lobe was resected to achieve 90% hepatectomy. Glisson’s pedicles to each lobe except the right lateral lobe were ligated and divided. According to the ischemic color change on the liver surface, hepatic parenchyma was transected using the clamp crushing technique. After performing 70% hepatectomy, the lateral half of the right lateral lobe was transected, paying attention not to damage the hepatic inflow and outflow. Glisson’s pedicle to the right lateral lobe was temporarily clamped to reduce bleeding during parenchymal transection.

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Table 1. Antibodies used in western blot analysis

Antigen	Company, serial number	Dilution
PCNA	Cell Signaling, 2586	1:2,000
SOCS3	Santa Cruz Biotechnology, sc-9023	1:5,000
Total-STAT3	Fitzgerald, 70R-50433	1:1,000
Phospho-STAT3	LifeSpan BioSciences, LS-C352904/70254	1:1,000
GAPDH	Novus Biologicals, NB300-221	1:10,000

PCNA, proliferating cell nuclear antigen; SOCS3, suppressor of cytokine signaling 3; STAT3, signal transducer and activator of transcription 3; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Portal pressure measurement and biochemical analysis

Portal pressure was measured via direct puncture using a 24-gauge needle connected to an invasive pressure monitoring device (Vigileo Monitor, Edwards Lifesciences, Irvine, CA, USA) before administration of terlipressin, 30 min, 1 h and 6 h after hepatectomy, and on the seventh postoperative day before sacrifice. Blood samples were collected from the femoral vein before administering terlipressin, 1 h and 6 h after hepatectomy, and on the seventh postoperative day. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin levels were measured using a biochemical analyzer (Chemical analyzer AU5800, Beckman Coulter Inc., Brea, CA, USA), while prothrombin time (PT) was measured using automated coagulation analyzer (Blood Coagulation Diagnosis analyzer, Diagnostica Stago Inc., Parsippany, NJ, USA).

Enzyme-linked immunosorbent assay (ELISA)

Interleukin 6 (IL-6), hepatocyte growth factor (HGF), and endothelin-1 (ET-1) serum levels were measured using commercially available ELISA kits (IL-6; Porcine IL-6 Quantikine ELISA Kit, P6000B, R&D systems, USA) (HGF; Pig hepatocyte growth factor, HGF ELISA Kit, CSB-E06795p, CUSABIO, China) (ET-1; Endothelin-1 Quantikine ELISA Kit, DET100, R&D systems, USA). Blood samples kept in the BD Vacutainer SST tube (Becton Dickinson, Franklin Lakes, NJ, USA) were centrifuged at 3,500 rpm for 10 min at 4°C to obtain serum. Serum samples were stored at -80°C. All procedures were performed according to the manufacturer's protocol. Briefly, 50 µl of 1:5 each diluted sample was placed in an antibody-coated well filled with 50 µl of assay diluent and incubated for 1 h at 37°C. Wells were washed three times with

PBS. The reaction was stopped by adding 50 µl of stop solution. Optical density at 450 nm was determined using a microplate reader.

Western blot

Suppressor of cytokine signaling 3 (SOCS3), total signal transducer and activator of transcription 3 (total-STAT3), phospho-STAT3, and proliferating cell nuclear antigen (PCNA) protein expression were examined using western blot. After mixing the protease inhibitor cocktail (Sigma Aldrich) in RIPA buffer, 1 mL of the mixture was added per tissue sample, and the tissue was smashed with a biomasher. Samples were centrifuged at 15,000 rpm for 15 min at 4°C, and the supernatants were sonicated four times with 30 s bursts. Samples were re-centrifuged at 15,000 rpm for 15 min at 4°C, and the supernatant was used for western blot. According to the manufacturer's protocol, 20 µg protein from each tissue sample was quantified using BCA protein assay and used for the experiment. As shown in **Table 1**, the primary antibody was diluted in 1X TBST with 5% skim milk and incubated overnight at 4°C. Furthermore, secondary antibodies (mouse anti-rabbit IgG-HRP, sc-2357, Santa Cruz Biotechnology and peroxidase anti-mouse IgG (H+L), PI-2000, Vector Laboratories) were all diluted at 1:1000 in the blocking solution (1X TBST with 5% skim milk solution) and incubated at room temperature for 1 h. Intensity for each protein was quantified using Image J (National Institutes of Health, Bethesda, USA). Data were normalized to the reference protein glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression.

Sinusoidal hemorrhage and Ki-67 immunohistochemical staining

Liver tissues were fixed in 10% formalin and stained with hematoxylin and eosin to quantify the area with sinusoidal hemorrhage (%). Samples were also prepared for immunohistochemistry with Ki-67. Tissue samples were cut into 4 µm sections and mounted on glass slide. After rehydration of sections in graded ethanol solutions, slides were blocked in 3% H₂O₂ solution for 10 min at room temperature. Slides were then boiled in 1X sodium citrate buffer in the microwave for 10 min for antigen retrieval.

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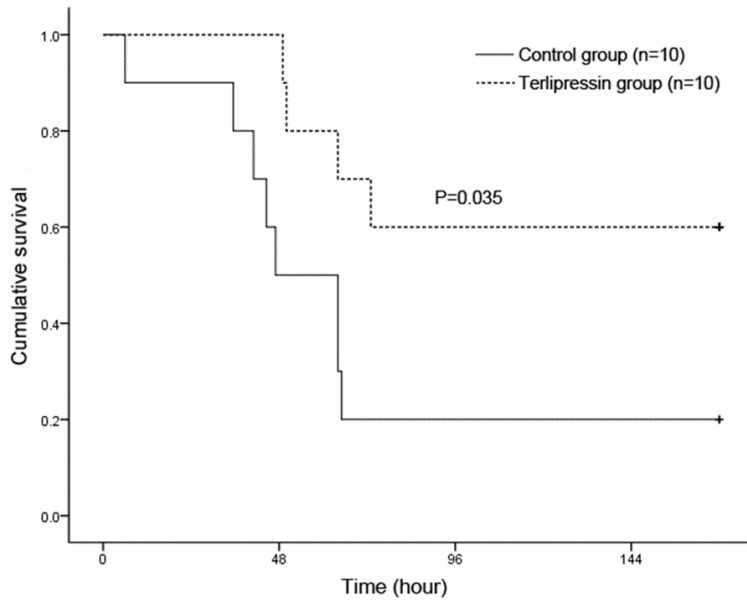
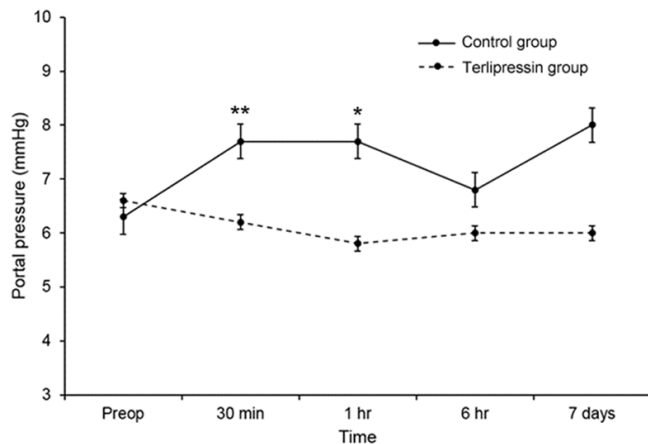


Figure 2. Cumulative survival curves in each group after 90% hepatectomy. The 7-day survival was 20% in the control group and 60% in the terlipressin group ($P = 0.035$).



Number at risk	Preop	30 min	1 hr	6 hr	7 days
Control group	10	10	10	10	2
Terlipressin group	10	10	10	10	6

Figure 3. Portal pressure change in each group after 90% hepatectomy. The portal pressure of the terlipressin group was lower than that of the control group at 30 min after hepatectomy with a borderline significance (6.2 vs. 7.7 mmHg, $P = 0.052$) and a notable significance 1 h after hepatectomy (5.8 vs. 7.7 mmHg, $P = 0.035$). Dots indicate means, and whiskers indicate the standard error of means. * $P < 0.05$ and ** $P < 0.1$ vs. control group at the same time point.

Slides were fixed with diluted (1:1000) anti-Ki-67 antibodies (Ki-67/MKI67 Antibody, NB-500-170, Novus Biologicals, USA) and incubated with normal blocking solution overnight at 4°C in a humid tray. Slides were incubated with

biotinylated secondary antibody in a normal blocking solution for 1 h at room temperature in a humid tray on the next day. Sections were then incubated with Avidin/Biotin blocking solution (VECTASTAIN ABC Kit (Mouse IgG), PK-4002, Vector Laboratories, USA) for 30 min at 37°C in a humid tray. DAB (3,3'-diaminobenzidine) solution (DAB Peroxidase Substrate Kit, SK-4100, Vector Laboratories, USA) was added to the sections. Sections were counterstained in Mayer's hematoxylin (Hematoxylin Solution, Mayer's, MHS16, Sigma Aldrich, St. Louis, MO, USA) until the desired degree of staining was achieved and washed with 1X PBS to stop further reaction. The slides were then scanned using a slide scanner (AxioScan Z1, Carl Zeiss Microscopy GmbH, Germany), and 10 photos were taken from each slide with the same magnification using ZEN 2 software (blue edition; Zeiss, Jena, Germany). The number of Ki-67-positive hepatocytes was determined in each photo.

Statistical analysis

All continuous values were presented as mean with standard deviation or median with interquartile range. Cumulative survival curves were analyzed using the Kaplan-Meier method and compared using the long-rank test. Continuous variables were compared between two groups using paired Student's *t*-test or Mann-Whitney U test as appropriate depending on the normality of data distribution. $P < 0.05$ was considered

to denote statistical significance, and P between 0.05 to 0.1 was deemed to be borderline significant. All statistical analyses were performed using SPSS software (version 24.0, IBM Corp., Armonk, NY, USA).

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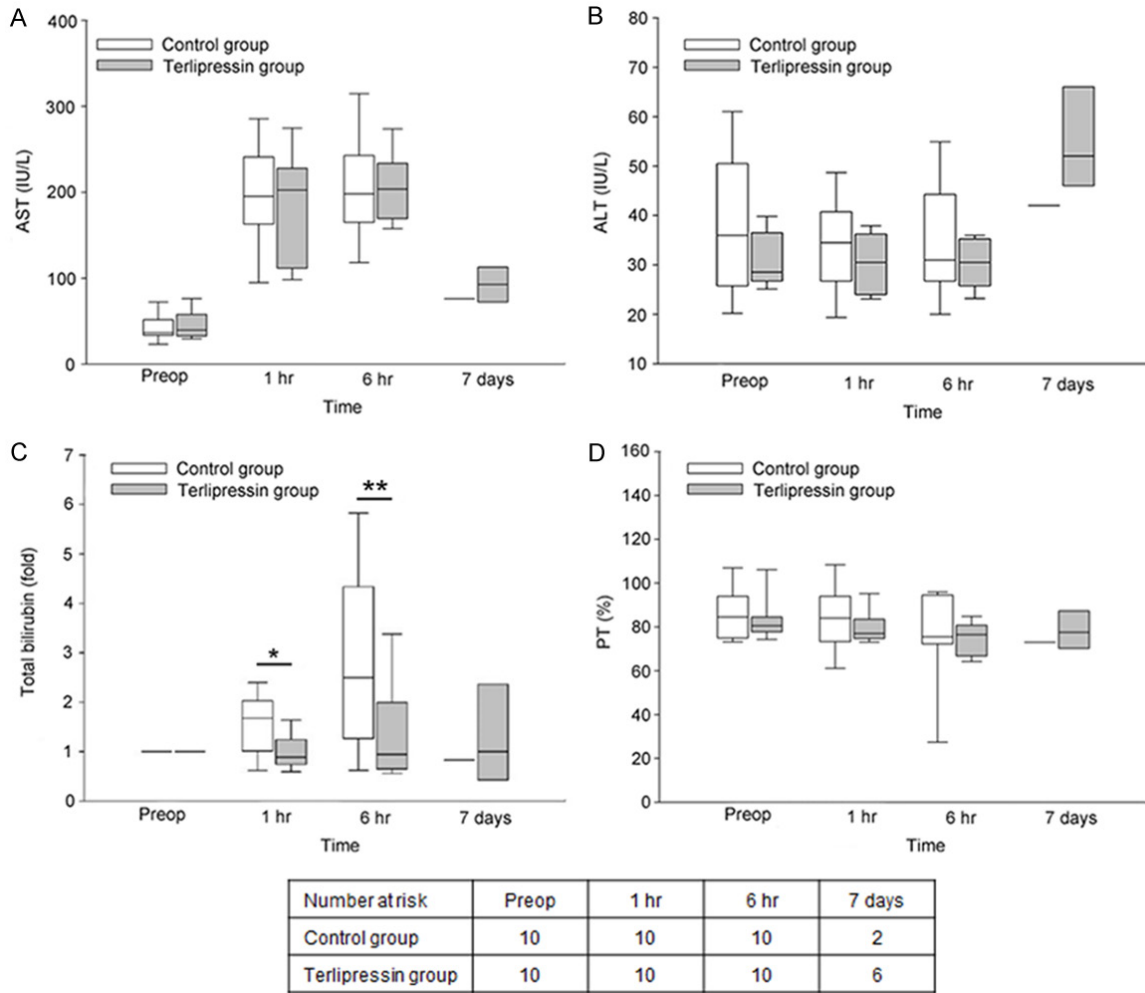


Figure 4. Postoperative evolutions of serum aspartate aminotransferase (AST) (A), alanine aminotransferase (ALT) (B), total bilirubin (C), and prothrombin time (D). Total bilirubin levels were demonstrated as fold changes over the preoperative values due to individual differences in preoperative value. Data are expressed as the median, with the 25-75% percentiles in boxes and the 5-95% percentiles as whiskers. * $P < 0.05$ and ** $P < 0.1$ vs. control group at the same time point.

Results

Survival

The terlipressin group showed higher 7-day survival rates than the control group (60% vs. 20%, $P = 0.035$, **Figure 2**). Most deaths occurred within 72 h after hepatectomy in both groups (three of four pigs in the terlipressin group and all eight pigs in the control group). The median survival time was 124 (49-168) h in the terlipressin group and 55 (6-128) h in the control group ($P = 0.043$). Autopsies were performed for all dead pigs to examine causes of death from potential surgical complications such as bleeding, but there was no demonstrable reason.

Portal pressure

Portal pressure measured before hepatectomy was comparable between the terlipressin and control groups (6.6 ± 1.1 vs. 6.3 ± 0.8 mmHg, $P = 0.631$) (**Figure 3**). In the control group, portal pressure increased 30 min after hepatectomy than that before hepatectomy with a borderline significance (6.3 ± 1.1 vs. 7.7 ± 2.1 , mmHg, $P = 0.083$). Portal pressure in the terlipressin group tended to be lower than that in the control group at all time points after hepatectomy. The terlipressin group showed lower portal pressure than the control group at 30 min after hepatectomy with a borderline significance (6.2 ± 0.9 vs. 7.7 ± 2.1 mmHg, $P = 0.052$), and a nota-

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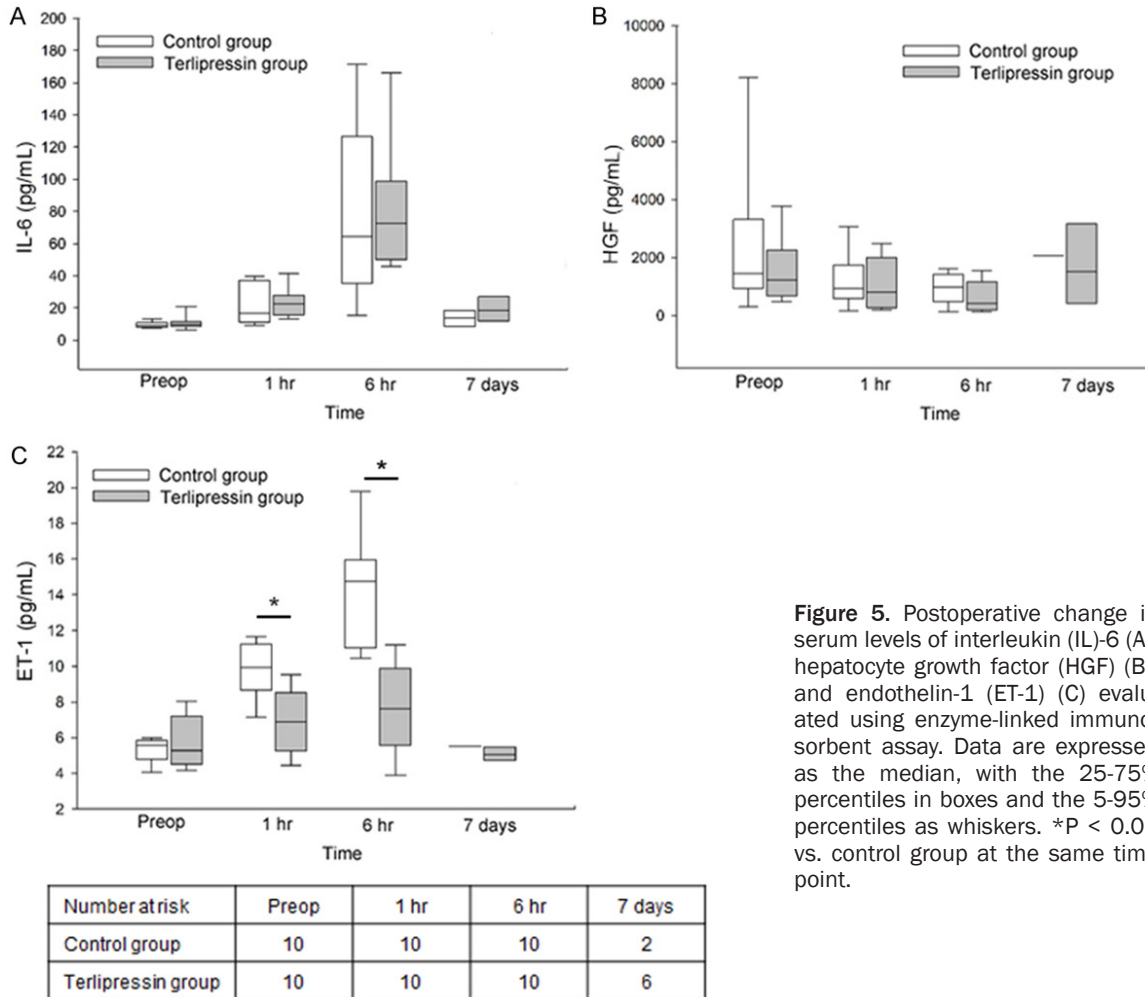


Figure 5. Postoperative change in serum levels of interleukin (IL)-6 (A), hepatocyte growth factor (HGF) (B), and endothelin-1 (ET-1) (C) evaluated using enzyme-linked immunosorbent assay. Data are expressed as the median, with the 25-75% percentiles in boxes and the 5-95% percentiles as whiskers. *P < 0.05 vs. control group at the same time point.

ble significance 1 h after hepatectomy (5.8 ± 1.1 vs. 7.7 ± 2.2 mmHg, $P = 0.027$).

Biochemical analysis

Serum AST, ALT, total bilirubin, and PT levels were evaluated to assess the extent of liver injury and functional status after extensive hepatectomy (**Figure 4**). In terms of serum AST, ALT, and PT levels, no significant differences were observed between both groups at all time points. Total bilirubin levels were demonstrated as a fold change over the preoperative value due to individual differences in preoperative values. Total bilirubin level in the terlipressin group was significantly lower than that in the control group 1 h after hepatectomy (1.00 vs. 1.57 fold change, $P = 0.023$), and at 6 h after hepatectomy, with a borderline significance (1.42 vs. 2.79 fold change, $P = 0.052$).

Protein expression by ELISA and western blot

IL-6 and HGF are known to promote liver regeneration after hepatectomy, and ET-1 is a marker of liver injury with vascular endothelial damage [15]. IL-6 and HGF serum levels evaluated by ELISA showed no significant difference between the terlipressin and control groups. However, ET-1 level was significantly lower in the terlipressin group than that in the control group at 1 and 6 h after hepatectomy ($P = 0.002$ and $P < 0.001$, respectively) (**Figure 5**).

Total-STAT3 and phospho-STAT3 play an important role in the process of liver regeneration, whereas SOCS3, one of the target genes of STAT3, functions as a negative regulator of liver regeneration [16-18]. There were no significant differences in total-STAT3, phospho-STAT3, and SOCS3 between the two groups as shown by

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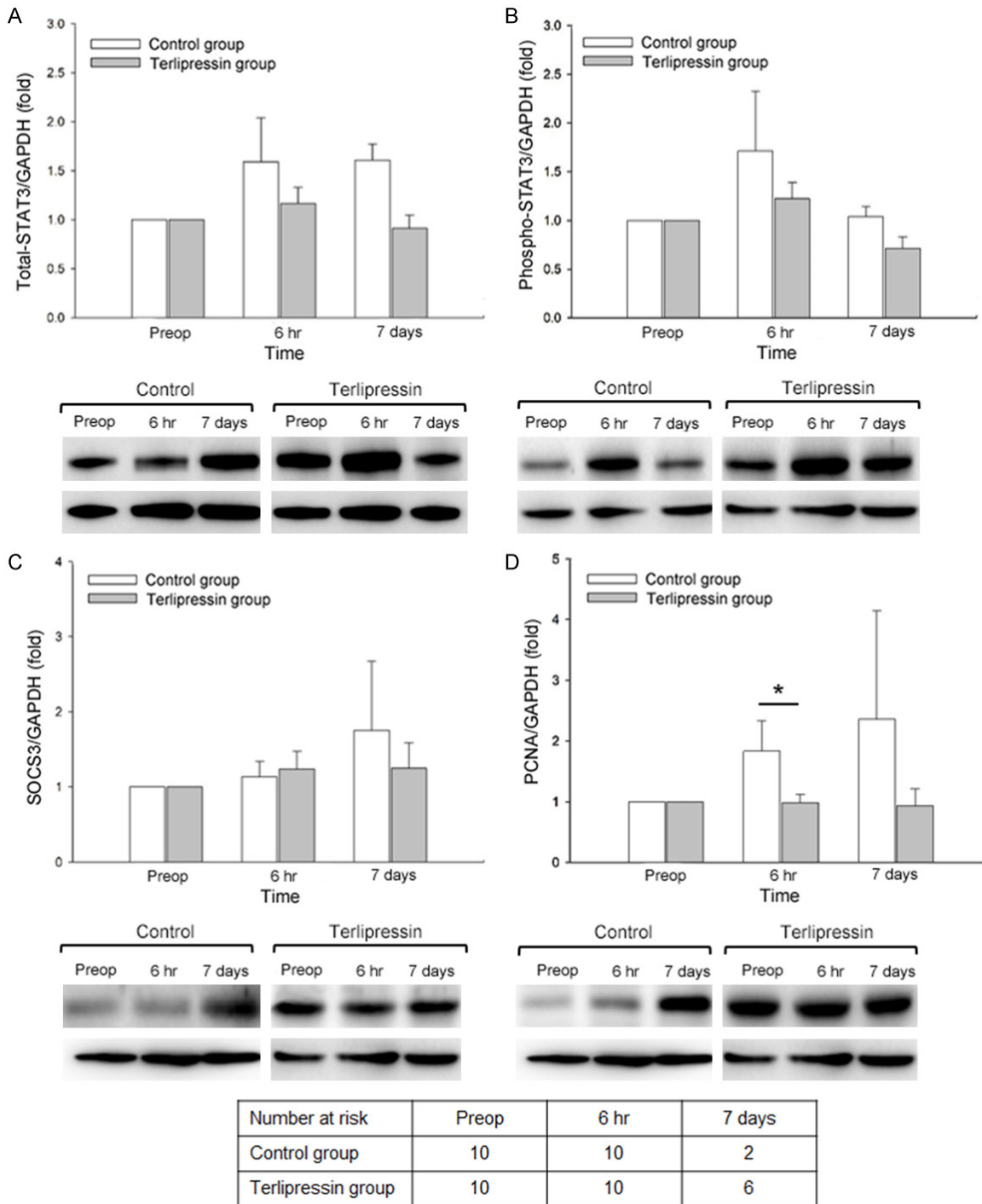


Figure 6. Postoperative change in protein expressions of total signal transducer and activator of transcription 3 (total-STAT3) (A), phospho-STAT3 (B), suppressor of cytokine signaling 3 (SOCS3) (C), and proliferating cell nuclear antigen (PCNA) (D). Representative images are demonstrated below each figure. Data were normalized to the reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and expressed as means and standard error. *P < 0.05 vs. control group at the same time point.

western blot. However, PCNA expression was higher in the control group than that in the terlipressin group at 6 h after hepatectomy

(P = 0.043) (**Figure 6**). PCNA reflects cell proliferation activity in the regenerating liver [19].

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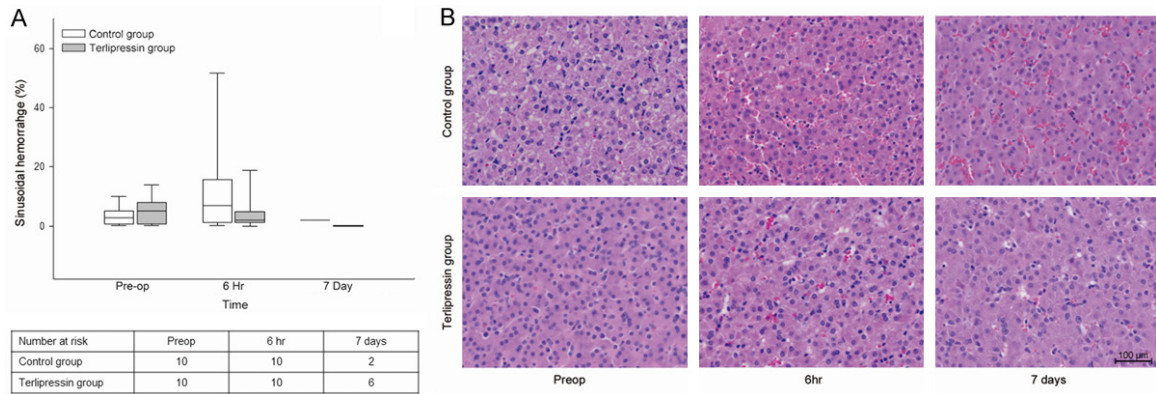


Figure 7. Postoperative change in the proportion of sinusoidal hemorrhage in each group (A) and representative images (Hematoxylin-eosin stain, original magnification x400) (B). Data are expressed as the median, with the 25-75% percentiles in boxes and the 5-95% percentiles as whiskers.

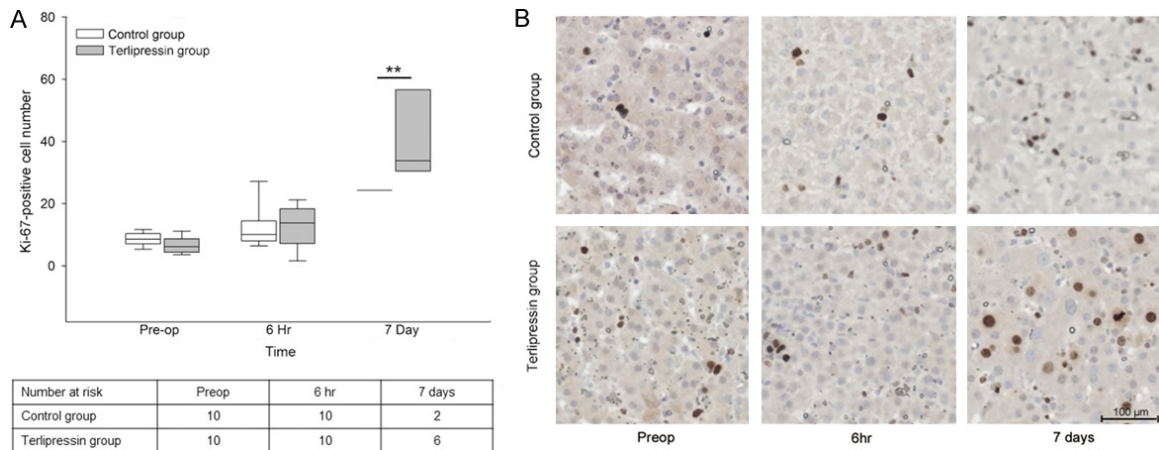


Figure 8. Postoperative change in Ki-67-positive cell number in liver tissues in each group (A) and representative images (original magnification x400) (B). Data are expressed as the median, with the 25-75% percentiles in boxes and the 5-95% percentiles as whiskers. ** $P < 0.1$ vs. control group at the same time point.

Sinusoidal hemorrhage and Ki-67

Sinusoidal hemorrhage area increased in the control group than in the terlipressin group 6 h after hepatectomy; however, the difference was not significant (**Figure 7A**). Representative images are shown in **Figure 7B**. The Ki-67-positive cell number in liver tissues was higher in the terlipressin group than that in the control group on the 7th postoperative day with a borderline significance ($P = 0.071$) (**Figure 8**).

Discussion

Recent advances in surgical techniques and perioperative management have enabled more extensive liver resection and transplantation with acceptable morbidity and mortality [20-

22]. However, PHLF remains a major cause of mortality that lacks an effective treatment, while SFSS continues to be a fatal complication after partial liver transplantation [23]. Several studies have reported that excessive portal pressure to the small remnant liver or graft plays a critical role in the development of both PHLF and SFSS [4, 24]. Therefore, various invasive procedures have been tried to prevent or treat these conditions. However, their effects on portal modulation are not only irreversible and unpredictable but also responsible for other severe complication [25]. Consequently, several studies have explored pharmacologic portal modulation as an alternative strategy. In addition to being non-invasive, drug dose and duration can be adjusted in pharmacologic por-

tal modulation depending on different clinical situations. In the current study, the portal modulation effects of terlipressin, a vasopressin analog, were identified. Terlipressin acts selectively on the V1a receptor in the portal venous system, resulting in splanchnic vasoconstriction with limited impact on systemic circulation [26]. Terlipressin is already safely used to treat hepatorenal syndrome and acute variceal bleeding [27, 28]. In a previous study conducted using rats subjected to 90% hepatectomy, terlipressin lowered portal pressure and promoted liver regeneration, resulting in the highest 1-week survival rate among various splanchnic vasoactive agents [29]. Therefore, the present study aimed to explore the clinical applicability of pharmacologic portal modulation by determining its effects using a large animal model while investigating its mechanism.

The 70% hepatectomy model has been used to identify pharmacologic portal modulation effects by resecting the liver as much as the subject could survive [13, 30]. In contrast, the 90% hepatectomy model has been used to investigate the effects of the pharmacologic intervention on liver regeneration and survival under extreme conditions wherein most subjects are expected to die [31]. In previous studies, portal pressure significantly increased after 90% hepatectomy in the porcine model, and all animals died within 51 h after hepatectomy [31, 32]. In the current study, portal pressure in the control group was higher at 30 min after hepatectomy than before hepatectomy, and the pressure was maintained during the study period. On the other hand, the terlipressin group showed lower portal pressure than the control group at 30 min and 1 h after hepatectomy. The portal modulation effect of terlipressin was rapid and highly effective, considering that only one injection immediately before hepatectomy induced the changes in portal pressure for 1 h after hepatectomy in the terlipressin group.

One of the most important effects of terlipressin was the optimized modulation of the timing and degree of the liver regeneration process. It was validated based on the trends in the expression of PCNA and Ki-67, which reflected cellular proliferation activity in the regenerating liver. PCNA expression significantly increased in the control group than that in the terlipressin group at 6 h after hepatectomy. However, the

proportion of Ki-67-positive cells was higher on postoperative day 7 in the terlipressin group than that in the control group. In the early stages after hepatectomy, quiescent hepatocytes enter the cell cycle (G0 to S phase), and cell division occurs to initiate liver regeneration [33]. Consequently, explosive cell division occurs as portal pressure and flow abruptly increase immediately after hepatectomy [34]. In contrast, the liver regeneration process in the terlipressin group occurred slowly; however, it was prolonged due to the portal modulation effect of terlipressin. Since dividing cells rarely function until normal microarchitectures are reformed, we assumed that well-controlled liver regeneration, especially in the early postoperative period, could be more favorable for the functional recovery of the liver [12, 35]. Furthermore, after liver resection, not only parenchymal cells, such as hepatocytes, but also non-parenchymal cells (e.g., Kupffer cells and hepatic stellate cells) proliferate for liver regeneration at different time points [36]. Hepatocytes begin cellular proliferation within 24 h, followed by biliary ductal cells, Kupffer cells, and hepatic stellate cells in the subsequent 2 days. Lastly, sinusoidal endothelial cells usually start active regeneration 4 days after hepatectomy [16]. Therefore, the portal modulation effect of terlipressin in slowing down the initial process of hepatocyte proliferation may balance and optimize cellular proliferation after extensive hepatectomy.

Cytokines activated during liver regeneration were also analyzed to determine the molecular mechanism underlying the effects of terlipressin on portal modulation. IL-6 is an inducer gene involved in liver regeneration that functions by binding to its receptors in the hepatocytes and promotes STAT 3 expression [37]. Activated STAT3 induces the expression of SOCS3, and activated SOCS3 arrests IL-6-induced STAT3 through negative feedback [17]. In the present study, IL-6 and SOCS3 levels showed no significant difference between the two groups. However, these genes were mostly involved in the early stages of liver regeneration, and other mechanisms, such as those associated with growth factors and metabolic pathways, could also affect liver regeneration collectively. Furthermore, the limitation of our study that experimental values were not measured in short intervals of time makes it difficult to identify serial changes over time.

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The portal modulation effect of terlipressin has led to reduced liver injury and improved survival. The expression of ET-1, a potent vasoconstrictive peptide, is activated by sinusoidal endothelial injury [15, 38]. In the current study, ET-1 levels were lower in the terlipressin group than those in the control group. Although not a statistically significant difference, taking into account the results of histological examinations showing consistently decreased degree of sinusoidal hemorrhage in the terlipressin group, these findings suggested that terlipressin could attenuate liver injury. Furthermore, total bilirubin levels in the terlipressin group were significantly lower than those in the control group. This could be due to the less endothelial injury in the terlipressin group as well as because terlipressin modulated the proportion of hepatocytes entering the cell cycle in the early postoperative period. Similar to previous studies, the study showed a high mortality rate (80%) in the control group within two days of performing 90% hepatectomy [39]. This result suggested the importance of early intervention after extensive hepatectomy. However, six pigs survived to 7 days in the terlipressin group, presenting a 7-day survival rate of 60%, which was three times higher than that of the control group.

A limitation of this study included the long-time interval of measurement between 6 h and 7 days after hepatectomy. If hemodynamic changes and liver generation marker levels had been examined using a shorter interval, the mechanism of terlipressin on portal modulation could have been understood more clearly. Further, if we identified the subcellular morphological changes between the groups, the portal modulation effects of terlipressin after extensive hepatectomy on the histologic aspect could be more clearly understood. Another limitation was that the effects of terlipressin at various doses and durations were not evaluated. Nevertheless, we confirmed the beneficial effect of terlipressin on portal modulation after extensive hepatectomy using a large animal model. Thus, this study could serve as the foundation of clinical trials for determining the effects of terlipressin on preventing or treating PHLF and SFSS.

Conclusion

Terlipressin rapidly modulated excessive portal pressure in the early postoperative period after

extensive hepatectomy in a large animal model. Consequently, the modulated portal pressure could optimize the liver regeneration process, resulting in reduced liver injury and improved survival.

Acknowledgements

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Disclosure of conflict of interest

None.

Abbreviations

PHLF, Post-hepatectomy liver failure; SFSS, Small-for-size syndrome; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; PT, Prothrombin time; IL-6, Interleukin 6; HGF, Hepatocyte growth factor; ET-1, Endothelin-1; SOCS3, Suppressor of cytokine signaling 3; Total-STAT3, Total signal transducer and activator of transcription 3; PCNA, Proliferating cell nuclear antigen; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase.

Address correspondence to: Dong-Sik Kim, Division of HBP Surgery and Liver Transplantation, Department of Surgery, Korea University College of Medicine, 73, Goryeodae-ro, Seongbuk-gu, Seoul 02841, Korea. Tel: +82-2-920-6620; Fax: +82-2-921-6620; E-mail: kimds1@korea.ac.kr

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