

Adverse effects of graft congestion and ameliorative effects of hepatocyte growth factor after liver transplantation in rats

ラット肝移植におけるグラフト肝うっ血の悪影響
と HGF の有効性

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Title:

Adverse effects of graft congestion and ameliorative effects of hepatocyte growth factor after liver transplantation in rats

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Conflicts of interest

Nothing to report.

List of abbreviations

ALT, alanine aminotransferase; HGF, hepatocyte growth factor; LML, left median lobe; LT, liver transplantation; NT, no treatment; POD, postoperative day; RML, right median lobe

Abstract

Living-donor liver transplantation and deceased-donor split-liver transplantation often result in congestion within liver grafts. The regenerative process and function of congested areas, especially graft congestion associated with liver transplantation, are not well-understood. Therefore, we created new rat models with congested areas in partially resected livers and orthotopically transplanted these livers into syngeneic rats to observe liver regeneration and function in congested areas. This study aimed to compare liver regeneration and the function of congested areas after liver resection and liver transplantation and to explore a new approach to ameliorate the adverse effects of graft congestion. Although the congested areas after liver resection regenerated normally on postoperative day 7, the congested areas after liver transplantation had poor regeneration with abscess development on postoperative day 7. Necrotic areas in congested areas were larger after transplantation than after resection on postoperative days 1, 3, and 7 ($p < 0.05$, $p < 0.05$, and $p < 0.01$, respectively). Although congested areas after liver resection did not affect survival, in the transplantation model, the survival of rats with congested areas was significantly poorer even with larger grafts than that of rats with smaller non-congested grafts ($p = 0.04$). Hepatocyte growth factor administration improved the survival rate of rats with congested grafts from 41.7% to 100%, improved the regeneration of congested areas, and significantly reduced the size of necrotic areas ($p < 0.05$). Thus, congested areas in liver grafts may negatively impact recipients. Short-term administration of hepatocyte growth factor may improve postoperative outcomes of recipients with graft congestion and contribute to more effective use of liver grafts (approval number: MedKyo-23137, Institutional Ethics Committee/Kyoto University).

INTRODUCTION

The increasing need for liver transplantation (LT) highlights the persistent issue of graft shortage.¹⁻³ Living-donor LT and deceased-donor split-LT are potential solutions to this shortage⁴⁻⁶; however, a notable problem associated with these partial LT procedures is the occasional risk of congested areas within grafts because the hepatic veins cannot be divided.^{7,8} Venous reconstruction is the only method to prevent congestion.⁹ However, depending on the graft type and anatomy, venous reconstruction can be complicated, and unintentional obstruction of reconstructed veins can occur; therefore, complete prevention of congestion can be difficult.¹⁰

Congested areas that occur after hepatic vein resection during liver resection can retain some function and undergo normal regeneration.¹¹ These areas rarely pose a major problem in clinical practice.¹²⁻¹⁴ In contrast, a few clinical studies reported poor outcomes associated with graft congestion, such as necrosis,^{8,15} poor function,^{8,15} and frequent recurrence of hepatocellular carcinoma.^{10,16} The function and regeneration of congested areas within grafts are not well-understood. Limited knowledge of graft congestion and approaches to improve its effects may negatively impact the outcomes of recipients and limit the effective use of grafts. Thus, the clarification of the function and regeneration of congested liver grafts and the development of new methods to improve the function of congested areas are warranted. Although experiments involving animal models can more effectively evaluate regenerative processes and functional outcomes of graft congestion, few relevant studies are available. Therefore, we created new rat models of congestion in partially resected livers and orthotopically transplanted those livers into syngeneic rats to observe liver regeneration and function in congested areas. This study investigated differences in the regeneration and function of congested areas after liver resection and LT. Furthermore, because of the low hepatocyte growth factor (HGF) serum levels, despite poor liver regeneration in congested areas after LT, we focused on the ability of HGF to promote liver regeneration¹⁷ and explored the effects of HGF administration on congested areas after LT.

PATIENTS AND METHODS

Animals

Male Lewis rats (weight, 250–320 g; Japan SLC Inc., Shizuoka, Japan) were housed under specific pathogen-free conditions in a temperature- and humidity-controlled environment with a 12-h light/dark cycle. The rats were fed a standard diet (F-2; Oriental Bio Service,

Kyoto, Japan) and had access to tap water *ad libitum* before and after surgery. The experimental protocol was approved by the Institutional Ethics Committee of Kyoto University (approval number: MedKyo-23137) and complied with the guidelines of the *Guide for the Care and Use of Laboratory Animals*.

Congestion models

We created new rat models that included congested areas after liver resection (Figure 1A). The model of congestion in the right median lobe (RML) was created by ligating the right hepatic vein, resulting in congested area in approximately 7% of the entire liver. The 30% residual liver (the RML) model with ligation of the right hepatic vein was defined as the 30% (7%) model. The 30% residual liver (the RML) model without vein ligation was defined as the 30% (0%) model (control model). In this study, the percentage outside parentheses represents the residual liver volume relative to the whole liver, and the percentage inside parentheses represents the congested area volume (Figure 1A).

The model of congestion in the left median lobe (LML) was created by ligating the left hepatic vein, resulting in congested area in approximately 10% of the whole liver. The 40% residual liver (the RML and LML) model with ligation of the left hepatic was defined as the 40% (10%) model (Figure 1A). Detailed instructions for creating these models are provided in SDC, Materials and Methods.

Additionally, the 50% residual liver (the RML and right lobe) model with ligation of the right hepatic vein was defined as the 50% (7%) model. The 60% residual liver (the RML, LML, and right lobe) model with ligation of the left hepatic vein was defined as the 60% (10%) model. These models were used for transplantation experiments (Figure 1B).

Experimental protocols

Summaries of the experiments are provided in Figure 1.

Experiment 1: Liver resection model

Five specimens were collected from each of the aforementioned 30% (0%), 30% (7%), and 40% (10%) models on postoperative day (POD)1, POD3, and POD7 (Figure 1A). Blood samples were collected from the inferior vena cava, and liver tissue was collected from congested and non-congested areas in the RML.

Experiment 2: LT model

Orthotopic LT was performed in syngeneic rats using livers from the 30% (0%), 30% (7%), 40% (10%), 50% (7%), and 60% (10%) models (Figure 1B). The procedure was performed in accordance with previously reported methods.^{18,19} Hepatic artery reconstruction was performed using a modified sleeve technique.²⁰ The hepatic vein was ligated on the back table, and congested areas were kept completely refluxed and filled with the histidine-tryptophan-ketoglutarate solution. The graft was chilled at 4 °C for 1 h. The details of the LT procedure are described in SDC, Materials and Methods and SDC, Video. Five specimens were collected on POD1, POD3, and POD7.

Experiment 3: LT plus HGF treatment model

The 50% (7%) model rats received recombinant human HGF (50 µg/mL; catalog number 294-HGN; R&D Systems, Minneapolis, MN, USA) administered at 100 µg/kg through the penile vein under anesthesia immediately after LT and on POD1 and POD2 (Figure 1C). The 50% (7%) model with HGF administration was defined as the 50% (7%) HGF model, and the 50% (7%) model without HGF administration, which was also used in experiment 2, was defined as the 50% (7%) no treatment (NT) model. Five specimens were collected on POD1, POD3, and POD7.

Serum biochemistry measurements

Serum alanine aminotransferase (ALT) levels, total bilirubin levels, other biochemical parameters, and blood counts were measured using Hitachi 7700 (Hitachi High-Tech Co., Tokyo, Japan). Serum HGF levels were measured using the Quantikine ELISA kit (catalog number MHG00; R&D Systems) according to the manufacturer's protocol. The Bio-Plex multiplex system (Bio-Rad Laboratories, Hercules, CA, USA) and Bio-Plex 200 (Bio-Rad Laboratories) were used to assess serum proinflammatory cytokine and chemokine levels according to the manufacturer's instructions. Data were analyzed using Bio-Plex Manager 6.1 (Bio-Rad Laboratories).

Histological analysis

After euthanizing the rats, liver specimens were collected, fixed in buffered formalin, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and observed using a microscope. Hepatocyte proliferation was evaluated by staining deparaffinized hepatic

sections using a rabbit polyclonal anti-Ki-67 antibody (ab15580; Abcam, Cambridge, UK) to detect Ki-67. The Ki-67 labeling index was calculated based on the percentage of positive cells. The numbers of total cells and positive cells were calculated automatically using QuPath²¹ (version 0.4.3) in all fields of view and the same search conditions. Moreover, hepatocyte loss and percentage of necrotic areas were calculated using the entire sectioned field of view and QuPath.

Measurement of the ATP level in liver tissues

Both congested and non-congested areas in the RML in each sample were snap-frozen and stored at -80°C until subsequent assays were performed. ATP in liver tissue was measured using an AMERIC-ATP(T) kit (Applied Medical Enzyme Research Institute Co., Tokushima, Japan) according to the manufacturer's instructions.

Statistical analysis

Continuous variables are presented as mean $\pm$ standard deviation. The Shapiro–Wilk test was performed to confirm whether the data of the continuous variables were normally distributed. Comparisons between groups were performed using the unpaired t-test or Mann–Whitney U test. Comparisons between multiple models during experiment 1 were performed using one-way analysis of variance followed by Dunnett's multiple comparison test or the Kruskal–Wallis test followed by Dunn's test (compared with the 30% [0%] model as the control). Comparisons of multiple models during experiments 2 and 3 were performed using the Tukey–Kramer test or Dunn test. Survival was analyzed using the log-rank test. Statistical significance was set at p -values of < 0.05 . All statistical analyses were performed using Prism 9 (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

Experiment 1: Liver resection model

All rats in the 30% (0%), 30% (7%), and 40% (10%) models survived until POD7. Intra- and postoperative images of the macroscopic and microscopic findings of the congested areas (obtained using hematoxylin and eosin staining) are shown in Figure 2. In the 30% (7%) model, the boundary between the congested and non-congested areas was no longer discernible on POD7 (Figure 2A). Microscopic examination showed hepatocyte loss in the congested area on POD1; however, hepatocyte loss and necrosis were not observed on POD7,

and the congested area appeared completely normal (Figure 2A). In the 40% (10%) model, the macroscopic findings were almost normal on POD7; however, the congested area was slightly atrophic. The microscopic findings indicated that hepatocytes were present only around the portal vein in the congested area on POD1; however, they recovered to almost normal levels on POD7 (Figure 2B). No biliary injury was observed in the congested areas on POD7.

The serum ALT levels of the 30% (7%) and 40% (10%) models significantly increased on POD3 ($p < 0.05$); however, these levels improved to within normal limits in all models on POD7 (Figure 3A). The serum HGF levels of the 30% (7%) and 40% (10%) models on POD3 were significantly lower than those of the 30% (0%) model ($p < 0.01$ and $p < 0.001$, respectively; Figure 3B). Regarding the other serum results, only the platelet counts exhibited differences (SDC, Table 1). Serum total bilirubin levels were within the normal range for all groups (SDC, Table 1).

According to the Ki-67 immunostaining findings, many Ki-67-positive cells were observed in the 30% (0%) model on POD1. Compared with the 30% (0%) model, fewer positive cells were observed in the normal regions of the 30% (7%) and 40% (10%) models on POD1 (Figure 4A). The cell counts indicated that the percentages of Ki-67-positive cells in the normal regions of the 30% (7%) and 40% (10%) models were significantly lower than those of the 30% (0%) model on POD1 ($p < 0.001$ and $p < 0.05$, respectively; Figure 4B). In the congested areas of the 30% (7%) and 40% (10%) models, fewer positive cells were noted on POD1, whereas relatively more positive cells were noted on POD3 (Figure 4A, B). On POD7, there were no significant differences in liver weight, body weight loss, and liver weight per body weight between the groups (SDC Figure 1A–C).

Regarding the ATP level in the liver tissue, compared with the 30% (0%) model, the 30% (7%) and 40% (10%) models had significantly lower ATP levels in the normal regions on both POD1 ($p < 0.01$ and $p < 0.05$, respectively) and POD3 ($p < 0.05$ and $p < 0.05$, respectively; Figure 4C). In the 30% (7%) and 40% (10%) models, the ATP levels tended to be higher in congested areas than in normal areas on POD1 and POD3 (Figure 4C). On POD7, the ATP levels were similar in all models, regardless of whether the areas were normal or congested (Figure 4C).

Experiment 2: LT model

The cold ischemic time was 61.9 ± 5.8 min, the warm ischemic time (approximately the same as portal venous clamping phase) was 12.6 ± 1.6 min, and the operative time was 71.8 ± 7.3 min. The survival rate of the 30% (0%) model was 100%. By contrast, the survival rates of the 30% (7%), 40% (10%), and 60% (10%) models were poor, and sufficient specimens on POD3 and POD7 could not be collected (SDC, Figure 1D); therefore, the use of these models was discontinued midway through the study. Because the 50% (7%) model exhibited a significantly worse survival rate than the 30% (0%) model ($p = 0.04$; Figure 5A), 12 transplantation experiments were performed to collect five specimens on POD7.

Postoperative images of the macroscopic and microscopic findings of congested areas (obtained using hematoxylin and eosin staining) are shown in Figure 5B. The macroscopic findings did not indicate necrosis in the 30% (0%) model or necrosis in the normal areas of the 50% (7%) model. However, some congested areas in the 50% (7%) model exhibited necrosis and abscesses on POD7 (Figure 5B). A comparison of the congested areas in the 30% (7%) resection model and 50% (7%) transplantation model showed significantly larger necrotic areas in the transplantation model on POD1, POD3, and POD7 ($p < 0.05$, $p < 0.05$, and $p < 0.01$, respectively; Figure 5C).

The mean serum HGF level was significantly lower in the 50% (7%) model than in the 30% (0%) model on POD1 ($p < 0.01$; Figure 5D). Similarly, the mean platelet count was significantly lower in the 50% (7%) model than in the 30% (0%) model on POD1 ($p < 0.001$; SDC, Table 2). The levels of inflammatory cytokines such as interleukin-6 and interferon-gamma were higher in the transplantation model than in the resection model; however, in the transplantation model, there were no significant differences between the groups (SDC, Table 2). Serum total bilirubin levels were within the normal range for all groups (SDC, Table 2).

Experiment 3: LT plus HGF treatment model

The survival rate of the 50% (7%) HGF model was significantly better than that of the 50% (7%) NT model ($p = 0.04$; Figure 6A). The macroscopic findings of the 50% (7%) HGF model indicated that the congested area had recovered to nearly normal on POD7 (Figure 6B). The microscopic findings showed that although hepatocyte loss on POD1 was similar to that of the 50% (7%) NT model, nearly normal findings were observed for the 50% (7%) HGF model on POD7 (Figure 5B, 6B). On POD7, the 50% (7%) HGF model exhibited significantly fewer necrotic areas in the congested area than the 50% (7%) NT model ($p <$

0.05; Figure 6C).

Although the serum ALT level of the 50% (7%) NT model was significantly higher (even up to POD7) than that of the 30% (0%) model ($p < 0.05$), the serum ALT level of the 50% (7%) HGF model improved to within the normal range on POD7 (Figure 7A). The serum HGF level of the 50% (7%) HGF model remained significantly higher than that of the 50% (7%) NT model on POD1 and POD7 ($p < 0.01$ and $p < 0.01$, respectively; Figure 7B). On POD7, the white blood cell count of the 50% (7%) NT model was significantly higher than that of the 50% (7%) HGF model ($p < 0.05$). Hemoglobin and platelet levels were significantly higher in the 50% (7%) HGF model than in the 50% (7%) NT model on POD7 ($p < 0.01$ and $p < 0.001$, respectively; SDC, Table 2).

Compared with the 50% (7%) NT model, the 50% (7%) HGF model exhibited significantly greater graft weight gain ($p < 0.05$), significantly greater proportion of the RML containing congested area ($p < 0.01$), and significantly better postoperative weight gain relative to the preoperative weight ($p < 0.05$) on POD7 (Figure 8A–C).

The ATP level in the liver tissue of the 50% (7%) HGF model was significantly higher than that of the 50% (7%) NT model in both non-congested and congested areas (Figure 8D).

DISCUSSION

In this study, we created new rat models with congested areas in the livers and orthotopically transplanted those livers into syngeneic rats. These models enabled us to clarify the differences in the regeneration and function of congested areas after liver resection and LT and the adverse effects of graft congestion. HGF administration improved the function and regeneration of congested areas in the liver grafts.

Although congested areas normally regenerate in rats after liver resection, they may delay the regeneration of normal areas and impair liver function in normal areas. Although increased serum ALT levels and cell loss around the veins were observed in congestion models during the early postoperative period, the liver had regenerated close to normal on POD7. This process closely imitates that observed in humans in clinical practice.^{12,14,22,23} The lobulated congested area in the 40% (10%) model also regenerated normally after liver resection. This indicates low risk of necrosis in congested areas after liver resection, even with minimal intrahepatic venous collaterals. Additionally, the congested areas showed the same venous formation as the normal areas, suggesting that vascular remodeling had occurred in the congested areas. The congestion models had lower serum HGF levels, fewer Ki-67-positive

cells in normal regions, and lower ATP levels in normal areas, suggesting that the presence of congested regions suppresses the regeneration of normal areas and impairs the function of normal areas. A possible reason for these findings is that the presence of congested areas may increase the pressure in the portal vein even in normal areas,^{24,25} resulting in excessive shear stress,²⁶ as high-pressure arterial blood flows into the portal vein because of vein occlusion in congested areas, and the portal venous blood flows back in congested areas and flows into normal areas.^{24,25} In this model, the creation of congested areas increased the portal pressure, and decreased the platelet counts (SDC Figure 1E, Table 1). Thus, congested areas associated with liver resection regenerate normally; however, normal areas may be adversely affected by congested areas.

Congested areas in liver grafts may cause necrosis and should not be considered the same as those associated with liver resection. Furthermore, congested areas in liver grafts may negatively affect the recipient. We used the livers from congestion models as grafts and observed the effects of graft congestion after transplantation. Although there have been studies of animal models of whole or partial LT,^{27,28} to the best of our knowledge, this is the first study to involve the transplantation and evaluation of partial liver grafts with congested areas. Necrosis and abscesses of the congested areas were observed in some cases, and fewer Ki-67-positive cells were observed after LT than after liver resection, even in normal areas. Therefore, early postoperative liver regeneration may be delayed after LT, which may render the congestion irreversible. Additionally, it should be noted that the 50% (7%) model had a worse survival rate than the 30% model, even though the former had a larger normal (non-congested) volume than the latter. This finding suggests that the cause of death in the 50% (7%) model may be due to the adverse effects of congested area rather than small-for-size syndrome. The adverse effects are likely due to necrosis and abscess formation in congested area, and may also involve impaired function in normal areas caused by the presence of congested area, as observed in the resection model. This is because rats are highly resistant to infection and abscesses in congested areas were likely not the only cause of death. Although larger grafts generally result in better recipient outcomes, the results of this study suggest that increasing the graft volume by adding congested areas without venous reconstruction may negatively affect outcomes. Possible reasons for differences in the regeneration of congested areas after liver resection and LT include cold storage, ischemia-reperfusion injury,^{29,30} and prolonged warm ischemia time. In congested areas, the arterial blood provides blood flow, and the portal vein is the outflow pathway; therefore, arterial blood flow is important.^{24,25}

When arterial reconstruction was not performed, the congested area in grafts became ischemic and dissolved, and the hepatocytes disappeared completely (SDC, Figure 2A). Thus, even after reconstruction of the portal and hepatic veins and reperfusion during LT, no new blood flow will enter the congested areas until arterial reconstruction is complete. Therefore, the warm ischemic time of congested areas can be considerably longer than that of normal areas, and hepatocytes in congested areas can be considered more damaged. During LT (in contrast to liver resection), damage caused by cold storage, ischemia-reperfusion injury, and longer warm ischemia times, as well as by congestion itself, may result in poorer function, increased risks of necrosis and abscess formation in congested areas, and negative effects of graft congestion.

HGF administration can improve the function and regeneration of congested areas in liver grafts. HGF is a potent promoter of liver regeneration.¹⁷ Serum HGF levels were significantly lower in congestion models and congested areas regenerated poorly and necrosis developed after LT. Therefore, we hypothesized that sufficient serum HGF concentrations can improve regeneration, and we focused on HGF in our study. HGF is known to bind to c-Met and activate downstream factors that strongly promote liver regeneration.^{31,32} HGF administration has been reported to promote liver regeneration after hepatectomy or LT in rats.³³⁻³⁵ Our findings indicated that the short-term administration of HGF improved survival, regeneration of congested areas, and graft weight gain. Furthermore, HGF administration inhibited necrosis and abscesses in congested areas by promoting liver regeneration, and ALT levels and white blood cell counts normalized with HGF treatment. HGF has been reported to have various systemic positive effects on the heart, intestine, kidney, and other organs,³⁶⁻³⁸ which might have contributed to the improvement of survival. However, HGF primarily accumulates in the liver,³⁹ and the main reason for the better outcome is believed to be the improvement of liver regeneration in both normal and congested areas. HGF stimulates the hepatic progenitor cells that emerge in injured liver,⁴⁰ which may have enhanced liver regeneration, especially in congested areas. Promoting liver regeneration in congested areas prevents the necrotic regions from providing a scaffold for abscess formation. The reduction of abscess formation likely had the greatest impact on outcome improvement. In the HGF-treated group, there were few abscesses in congested areas, and the liver weight gain of the lobes containing congested areas was greater than in the no-treatment group. In contrast, the congested areas of the dead rats without HGF administration were almost completely necrotic, with only a few hepatocytes remaining around the portal vein (SDC Figure2B). The

HGF dose, timing, and duration were determined based on previous reports.^{33,35,41-43} The total dosage was similar to amounts previously reported to have improved liver regeneration after partial hepatectomy in rats.^{33,35} In addition, the daily dose in this experiment corresponds to the dose used in human clinical trials.⁴² Although continuous infusion is thought to be more effective because of its short half-life, intravenous injection was also reported to be effective,^{39,41,43} thus a simple intravenous injection was used in this study. HGF was administered for 3 days because liver regeneration after liver injury in rats peaks mainly within this period.^{44,45} All rats that died did so within 3 days after transplantation in this experiment, and long-term administration can cause adverse effects.^{42,43} Modifications of HGF administration, such as higher doses, longer courses, and continuous infusion, may result in additional benefits. Although extremely large doses were administered compared to endogenous HGF present in the blood, the results for serum HGF in Figure 7B reflect almost endogenous HGF levels due to HGF's short half-life.⁴² HGF administration may also increase endogenous HGF production. Graft congestion has been reported to increase the rate of hepatocellular carcinoma recurrence^{10,16}; however, the administration of HGF might reduce this rate by preventing abscesses and inflammation.⁴⁶ The safety of administering recombinant human HGF to humans has been confirmed.⁴² A similar drug, hepatocyte growth promoter factor, is used as a treatment in some countries⁴⁷; therefore, there are few obstacles to its clinical application. Future experiments with large animals that are more similar to humans should be conducted. If similar results are observed, the clinical application of HGF and related drugs can be implemented. Our results suggest that short-term HGF administration for cases of graft congestion can improve the outcomes of recipients and lead to effective use of marginal grafts.

This study has some limitations. First, differences in intrahepatic venous collaterals between rats and humans may have affected the results. Second, the possibility of rejection should be considered in clinical practice. However, this study used a syngeneic model; therefore, the effects of rejection could not be evaluated. Third, daily antibiotics were not administered during this experiment; however, their use may have resulted in reduced abscesses in congested areas of the grafts and improved outcomes of the congestion group. Fourth, only the surviving rats in the transplantation model that included congestion were analyzed; therefore, bias may have occurred. Finally, the mechanism of HGF action and the remodeling process in congested areas should be clarified in detail in future studies.

In conclusion, this study used congestion models of liver resection and LT to investigate the

effects of congested areas. Although congested areas regenerate normally after liver resection, they may negatively affect normal areas. Graft congestion associated with LT should not be equated with that associated with liver resection, and the possibilities of necrosis and abscesses should be considered. Congested areas of liver grafts may negatively affect recipients. Short-term administration of HGF can improve the function and regeneration of congested areas in liver grafts, improve postoperative outcomes, and lead to effective use of marginal grafts.

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CONFLICT OF INTEREST

The authors of this manuscript have no conflicts of interest (as described in the guidelines of *Liver Transplantation*) to disclose.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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FIGURE LEGENDS

Figure 1. Experiment summary. (A) Experiment 1 included the liver resection model. The 30% (0%), 30% (7%), and 40% (10%) models were created by liver resection and vein ligation. Gray color within the liver represents the congested area. (B) Experiment 2 included the liver transplantation model. The livers from the 30% (0%), 30% (7%), 40% (10%), 50% (7%), and 60% (10%) models were used as grafts for experiment 2. These grafts were chilled at 4 °C for 1 h in HTK solution and transplanted into syngeneic rats. (C) Experiment 3 included the liver transplantation plus HGF administration model. Rats in the 50% (7%) liver transplantation model received 100 µg/kg recombinant human HGF administered through the penile vein under anesthesia immediately after surgery and on POD1 and POD2.

Abbreviations: HGF, hepatocyte growth factor; HTK, histidine-tryptophan-ketoglutarate; IV, intravenous injection; LHV, left hepatic vein; LML, left median lobe; POD, postoperative day; human; RHV, right hepatic vein; RML, right median lobe.

Figure 2. Macroscopic and microscopic findings of the liver resection model. (A, B) Macroscopic and microscopic findings (hematoxylin and eosin staining) of congested areas observed intraoperatively and on POD1, POD3, and POD7 in the 30% (7%) model (A) and the 40% (10%) model (B). Abbreviation: POD, postoperative day.

Figure 3. Serological findings of the liver resection model. (A) Serum ALT levels. (B) Serum HGF levels. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Abbreviations: ALT, alanine aminotransferase; POD, postoperative day.

Figure 4. Ki-67 immunostaining findings and ATP levels in liver tissues of the liver resection model. (A) Ki-67 immunostainings of normal and congested areas on POD1 and POD3. (B) Percentage of Ki-67-positive cells in normal and congested areas in each model on POD1 and POD3. (C) ATP levels in normal and congested tissues in each model on POD1, POD3, and POD7. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Abbreviation: POD, postoperative day.

Figure 5. Results of experiment 2. (A) Survival rates of the 30% (0%) and 50% (7%) liver transplantation models. (B) Macroscopic and microscopic findings (hematoxylin and eosin staining) of congested areas on POD1, POD3, and POD7 in the 50% (7%) model. On POD7, part of the congested area was necrotic and abscessed (▲). (C) Comparison of the extent of

necrosis in the same congested areas in the 30% (7%) resection model and 50% (7%) transplantation model. (D) Serum HGF levels on POD1. $*p < 0.05$, $**p < 0.01$. Abbreviation: POD, postoperative day.

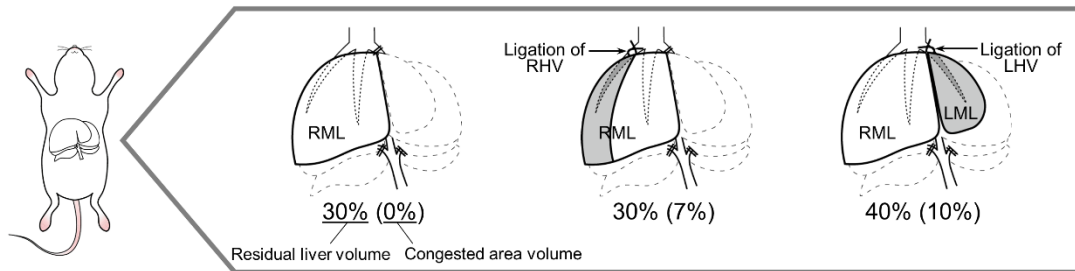
Figure 6. Results of experiment 3. (A) Survival rates of the 50% (7%) HGF model and 50% (7%) NT model (without HGF treatment). (B) Macroscopic and microscopic findings (hematoxylin and eosin staining) of the 50% (7%) HGF model. (C) Percentage of necrosis in the congested areas in the 50% (7%) HGF and 50% (7%) NT models on POD7. $*p < 0.05$. Abbreviations: HGF, hepatocyte growth factor; NT, no treatment; POD, postoperative day.

Figure 7. Serum ALT and HGF levels in liver transplantation models. (A) Serum ALT levels. (B) Serum HGF levels. $*p < 0.05$, $**p < 0.01$. Abbreviations: ALT, alanine aminotransferase; HGF, hepatocyte growth factor; NT, no treatment; POD, postoperative day.

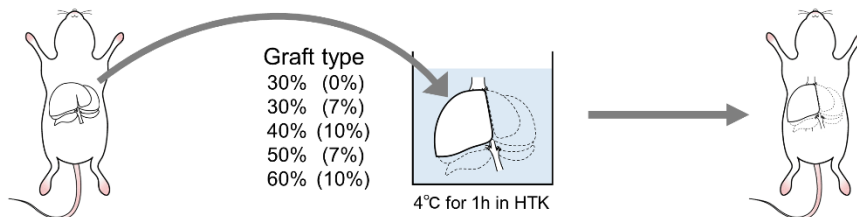
Figure 8. Comparison of the results of the 50% (7%) HGF model and 50% (7%) NT model (without HGF treatment). (A) Graft weight gain on POD7 compared with the preoperative weight. (B) Percentage of the right median lobe weight, including the congested area, compared with the whole graft weight on POD7. (C) Body weight ratio relative to the preoperative weight on POD7. (D) ATP levels in normal and congested areas of each model. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Abbreviations: HGF, hepatocyte growth factor; NT, no treatment; POD, postoperative day.

Figure 1

(A) Experiment 1: Liver resection model



(B) Experiment 2: Liver transplantation model



(C) Experiment 3: Liver transplantation plus HGF treatment model

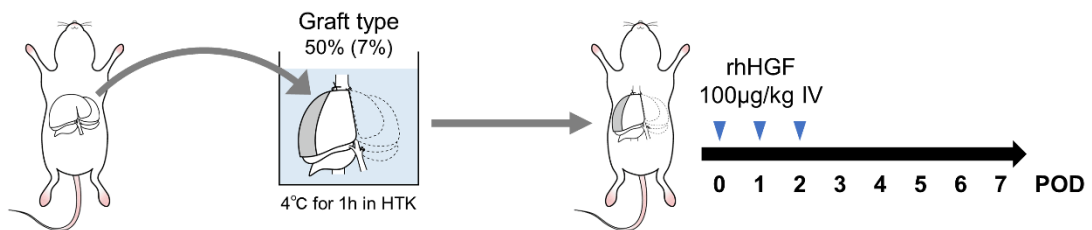


Figure 2

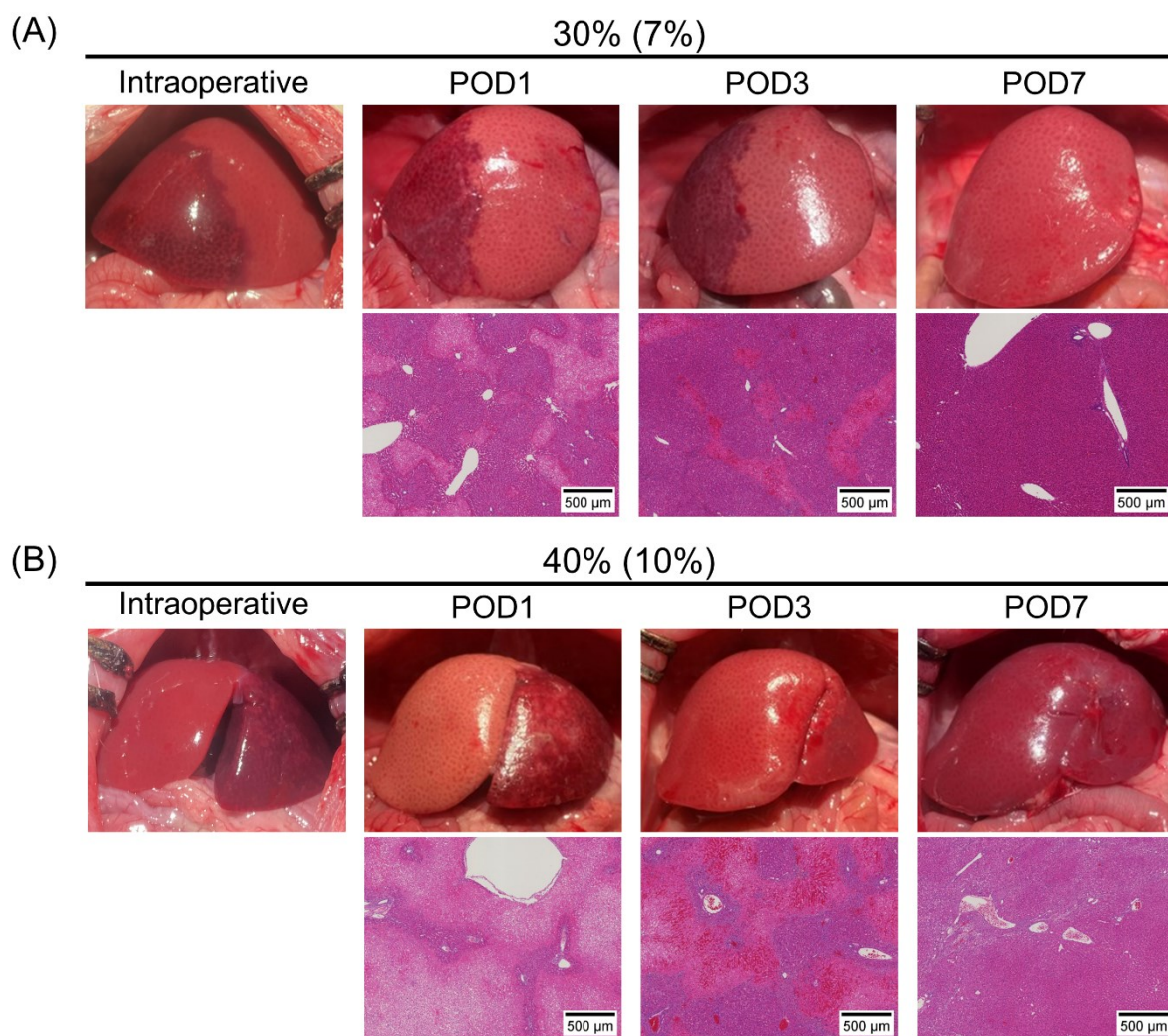


Figure 3

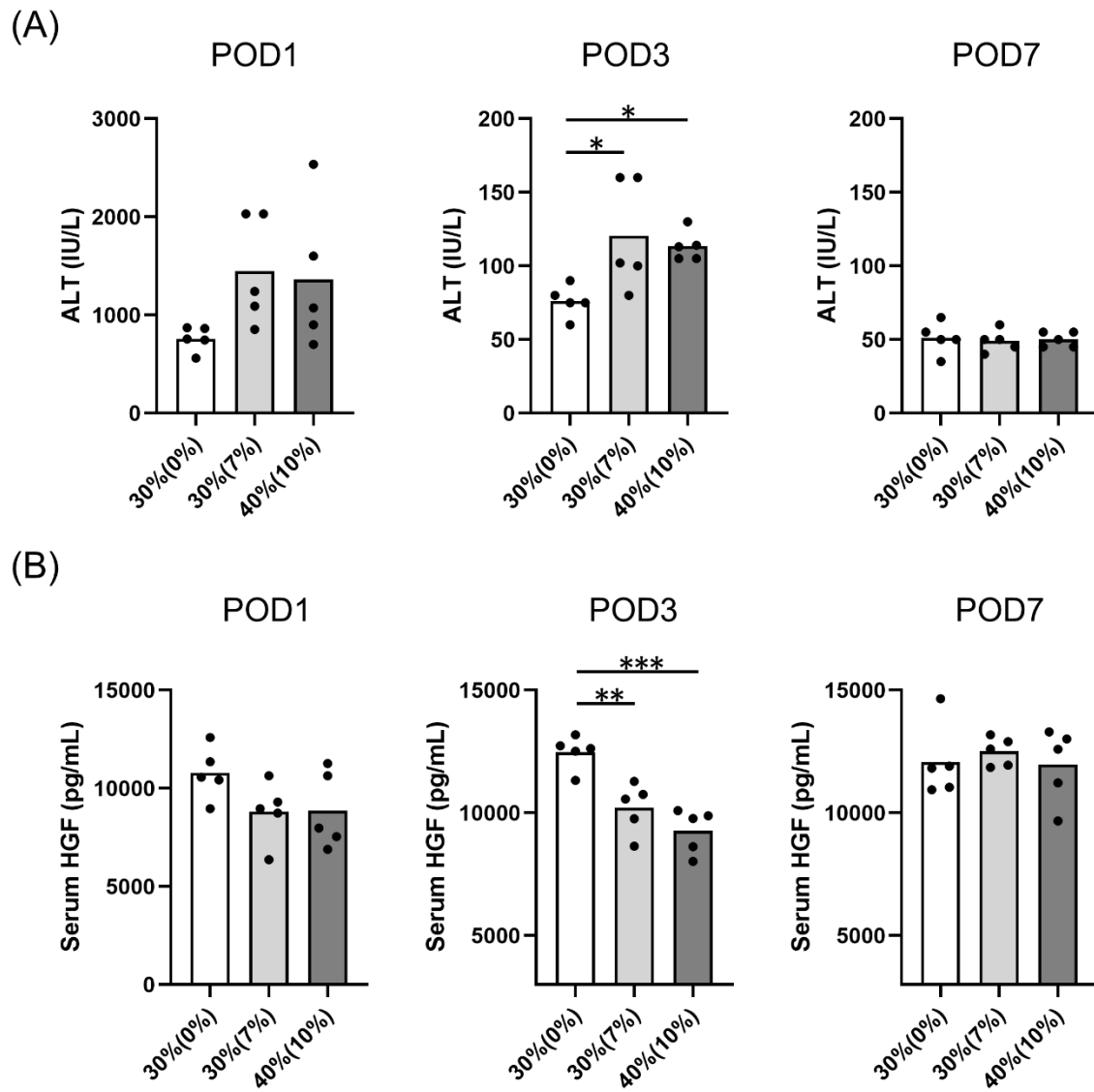


Figure 4

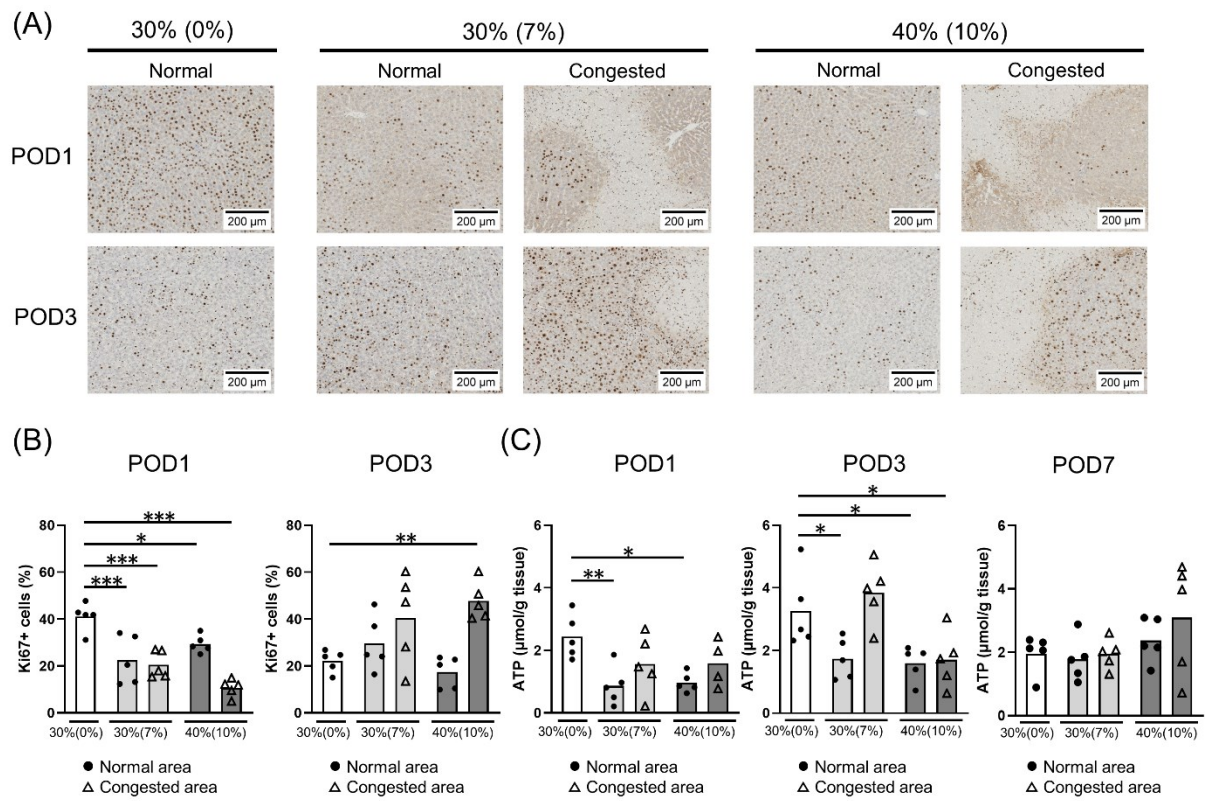


Figure 5

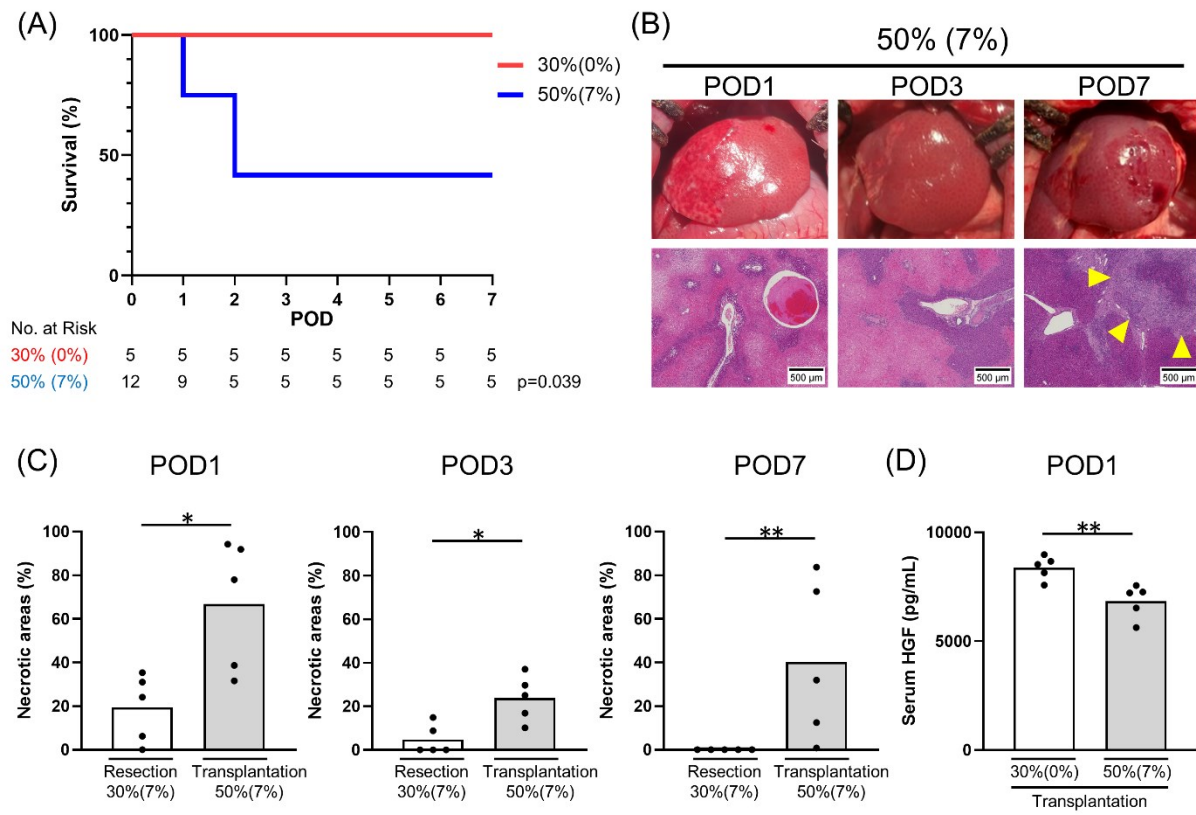
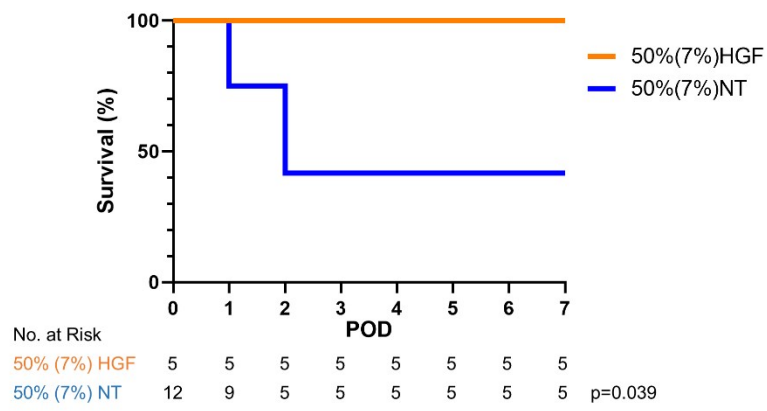
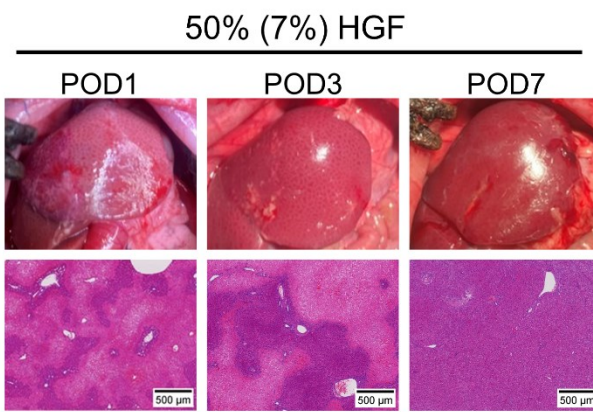


Figure 6

(A)



(B)



(C)

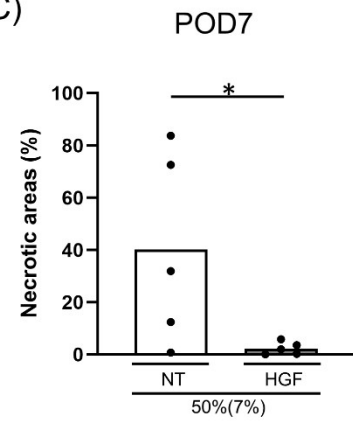


Figure 7

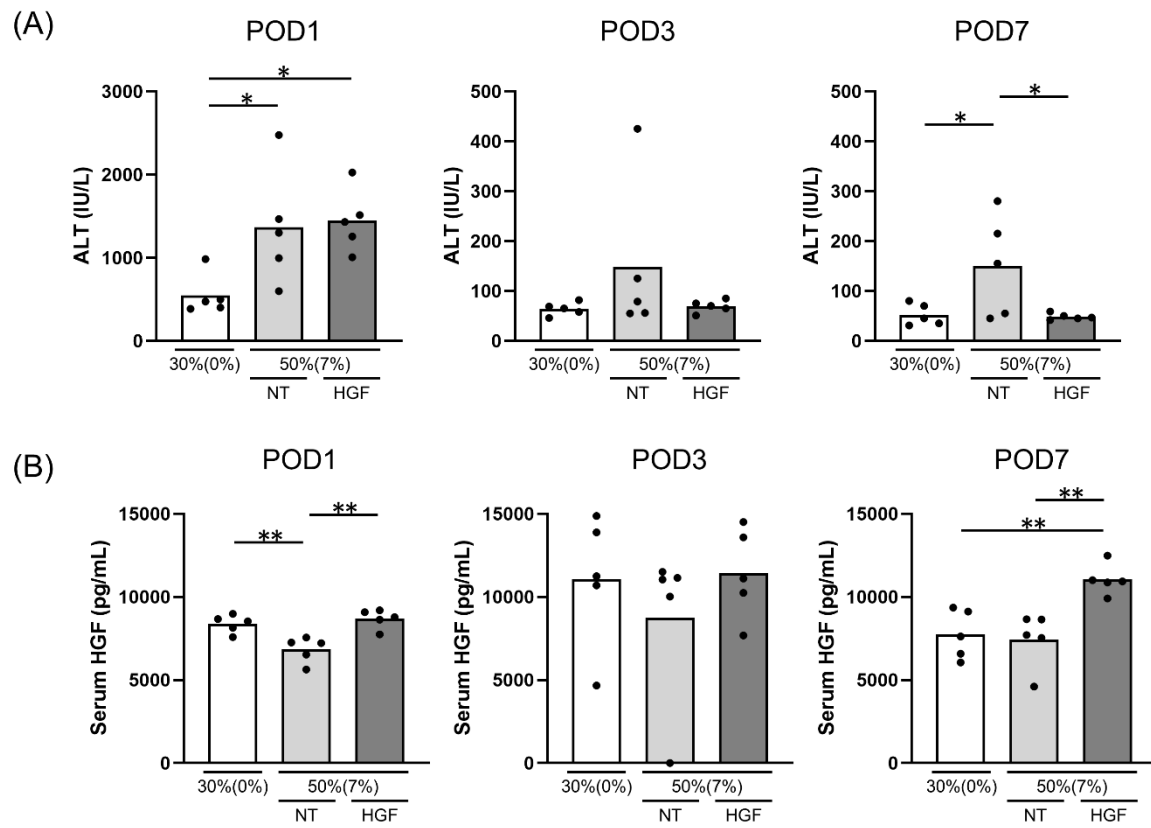
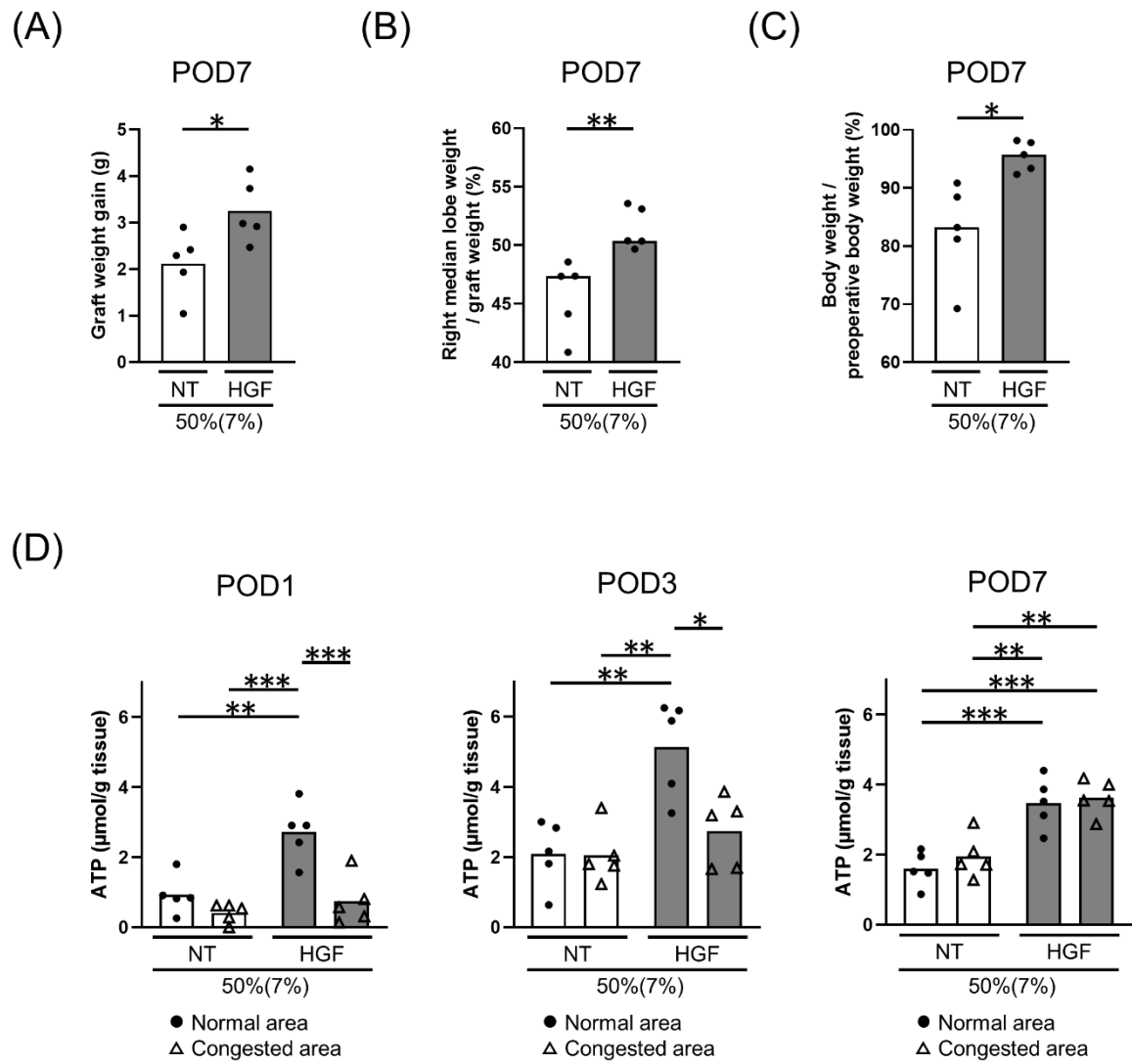
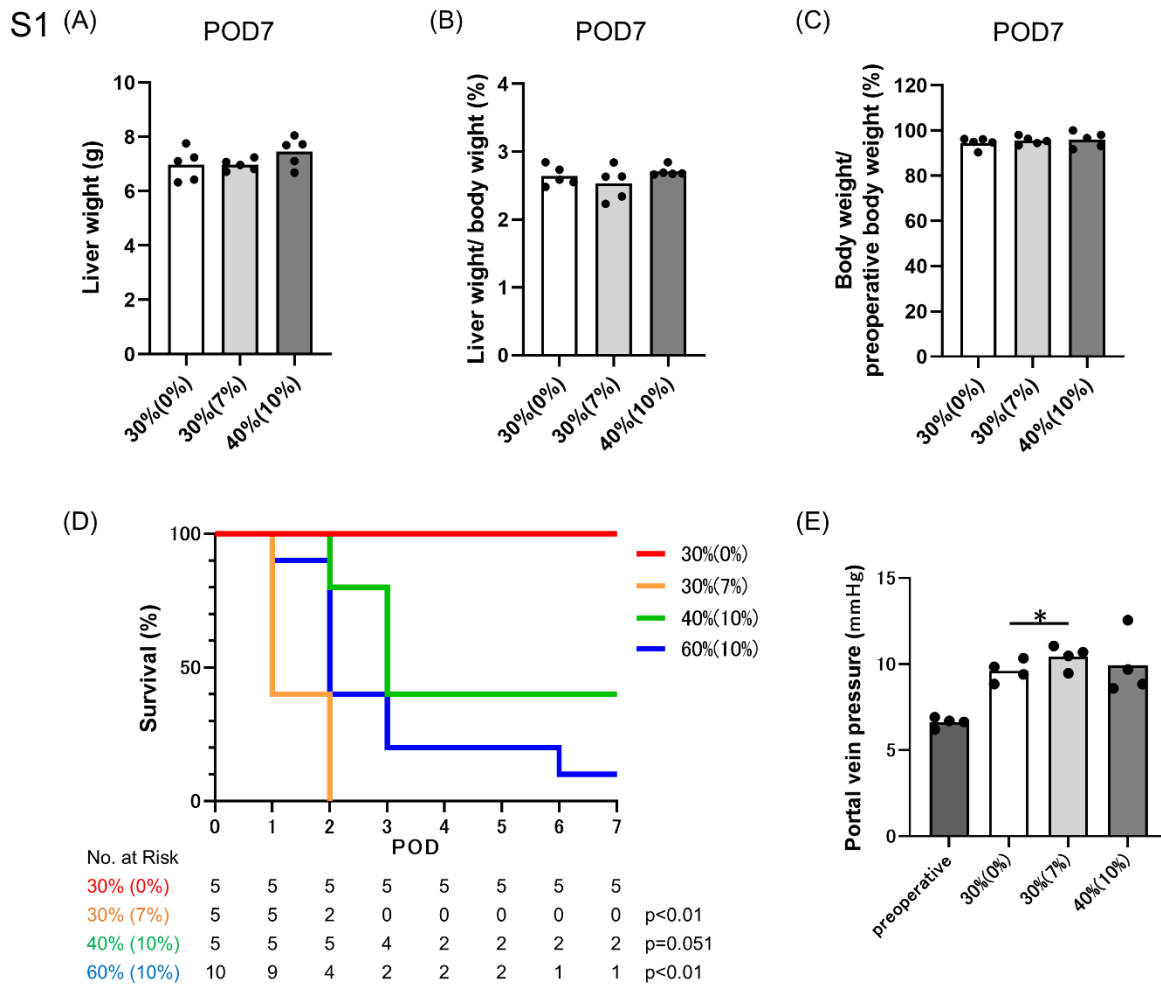


Figure 8



Supplemental Digital Content

SDC, Figure 1



(A) Liver weight on POD7 in resection models. There are no significant differences between the groups.

(B) Percentage of liver weight compared with body weight on POD7 in resection models. There are no significant differences between the groups.

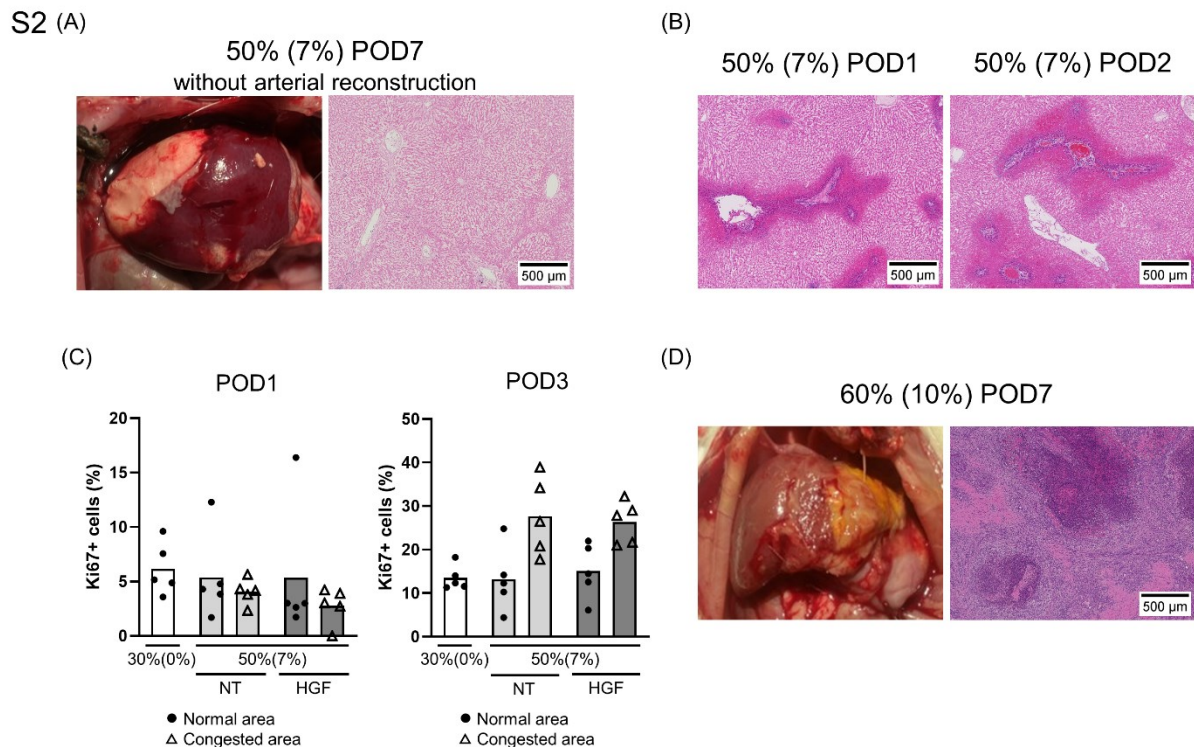
(C) Body weight ratio relative to the preoperative body weight on POD7 in resection models. There are no significant differences between the groups.

(D) Survival rates of the 30% (7%), 40% (10%), and 60% (10%) liver transplantation models. These models exhibit worse survival than the 30% (0%) control model ($p < 0.01$, $p = 0.051$, and $p < 0.01$, respectively).

(E) Portal vein pressure in the same rats in resection models. Portal vein pressure is significantly higher in the 30% (7%) model than in the 30% (0%) model.

* $p < 0.05$. POD, postoperative day.

SDC, Figure 2



(A) Macroscopic and microscopic findings (hematoxylin and eosin stain) of the congested area without arterial reconstruction in the 50% (7%) transplantation model on POD7. In the congested area, hepatocytes are completely shed, leaving only the cytoskeleton.

(B) Microscopic findings (hematoxylin and eosin stain) of the congested area of the dead rat in the 50% (7%) NT model on POD1 and POD2. The congested area is almost completely necrotic, with only a few hepatocytes around the portal vein.

(C) Percentage of Ki-67-positive cells in each region of the liver transplantation model on POD1 and POD3. Ki-67 immunostaining shows no significant differences in the percentages of Ki-67-positive cells between the 50% (7%) HGF and 50% (7%) NT models on POD1 and POD3.

(D) Macroscopic and microscopic findings (hematoxylin and eosin stain) of the congested area in the 60% (10%) transplantation model on POD7. The congested area is completely necrotic and abscessed.

HGF, hepatocyte growth factor; NT, no treatment; POD, postoperative day.

SDC, Table 1. Serum biochemical findings and cytokines in liver resection models

		30% (0%)	30% (7%)	40% (10%)
WBC (/ μ L)	POD1	5120 $\pm$ 449	4180 $\pm$ 726	5220 $\pm$ 963
	POD3	9980 $\pm$ 1283	10060 $\pm$ 2422	8760 $\pm$ 2440
	POD7	9880 $\pm$ 1057	8420 $\pm$ 2424	12425 $\pm$ 6663
Hb (g/dL)	POD1	14.1 $\pm$ 0.8	13.3 $\pm$ 0.9	13.6 $\pm$ 0.5
	POD3	13.5 $\pm$ 0.5	13.3 $\pm$ 0.3	13.2 $\pm$ 0.4
	POD7	12.9 $\pm$ 0.6	12.0 $\pm$ 2.1	13.2 $\pm$ 0.8
Plt (10 ⁴ / μ L)	POD1	61.6 $\pm$ 8.8	50.2 $\pm$ 7.7	47.6 $\pm$ 9.9*
	POD3	65.5 $\pm$ 6.3	58.7 $\pm$ 13.8	55.8 $\pm$ 5.6
	POD7	68.4 $\pm$ 6.4	60.5 $\pm$ 23.5	74.8 $\pm$ 5.4
AST (IU/L)	POD1	1110 $\pm$ 338	1629 $\pm$ 484	1781 $\pm$ 862
	POD3	252 $\pm$ 105	256 $\pm$ 97	326 $\pm$ 104
	POD7	228 $\pm$ 115	235 $\pm$ 168	257 $\pm$ 132
T-Bil (mg/dL)	POD1	0.18 $\pm$ 0.04	0.26 $\pm$ 0.13	0.26 $\pm$ 0.13
	POD3	0.20 $\pm$ 0	0.20 $\pm$ 0	0.20 $\pm$ 0
	POD7	0.20 $\pm$ 0	0.20 $\pm$ 0	0.20 $\pm$ 0
ChE (IU/L)	POD1	62.4 $\pm$ 11.7	51.6 $\pm$ 6.5	64.8 $\pm$ 9.9
	POD3	56.0 $\pm$ 14.7	59.6 $\pm$ 12.0	53.2 $\pm$ 14.6
	POD7	84.0 $\pm$ 26.1	99.0 $\pm$ 6.5	94.0 $\pm$ 4.2
γ GPT (IU/L)	POD1	2.2 $\pm$ 0.4	2.0 $\pm$ 0	2.2 $\pm$ 0.4
	POD3	2.6 $\pm$ 1.3	3.4 $\pm$ 1.5	2.6 $\pm$ 0.9
	POD7	2.6 $\pm$ 1.3	2.6 $\pm$ 1.3	2.6 $\pm$ 1.3
IL-6 (pg/mL)	POD1	710 $\pm$ 194	720 $\pm$ 103	798 $\pm$ 294
	POD3	689 $\pm$ 195	694 $\pm$ 70	642 $\pm$ 90
	POD7	1472 $\pm$ 1110	1595 $\pm$ 951	1943 $\pm$ 2363
IFN- γ (pg/mL)	POD1	887 $\pm$ 222	864 $\pm$ 79	989 $\pm$ 339
	POD3	846 $\pm$ 167	807 $\pm$ 106	729 $\pm$ 128
	POD7	1836 $\pm$ 1279	1740 $\pm$ 966	1638 $\pm$ 1697
TNF- α (pg/mL)	POD1	1856 $\pm$ 356	1957 $\pm$ 262	2074 $\pm$ 300
	POD3	1771 $\pm$ 321	1713 $\pm$ 592	2229 $\pm$ 386
	POD7	3016 $\pm$ 1134	3346 $\pm$ 1594	1844 $\pm$ 766
IL-1a (pg/mL)	POD1	614 $\pm$ 73	586 $\pm$ 71	595 $\pm$ 43
	POD3	724 $\pm$ 90	619 $\pm$ 79	629 $\pm$ 45
	POD7	1300 $\pm$ 702	1351 $\pm$ 722	960 $\pm$ 482
IL-1b (pg/mL)	POD1	359 $\pm$ 92	365 $\pm$ 87	274 $\pm$ 25
	POD3	1809 $\pm$ 872	1576 $\pm$ 915	1566 $\pm$ 830
	POD7	728 $\pm$ 374	790 $\pm$ 338	719 $\pm$ 447
VEGF (pg/mL)	POD1	179 $\pm$ 72	172 $\pm$ 62	101 $\pm$ 33
	POD3	1180 $\pm$ 778	919 $\pm$ 583	776 $\pm$ 697
	POD7	525 $\pm$ 283	654 $\pm$ 310	503 $\pm$ 376

Values are presented as mean $\pm$ standard deviation.

30% (0%) model vs. 40% (10%) model: $*p < 0.05$.

γ GPT: gamma-glutamyl transpeptidase; AST: aspartate aminotransferase; ChE: cholinesterase; Hb: hemoglobin; IFN- γ : interferon-gamma; IL-6: interleukin-6; IL-1a: interleukin-1 alpha; IL-1b: interleukin-1 beta; Plt: platelet; POD: postoperative day; T-Bil: total bilirubin; TNF- α : tumor necrosis factor alpha; VEGF: vascular endothelial growth factor; WBC: white blood cells.

SDC, Table 2. Serum biochemical findings and cytokines in liver transplantation models

		30% (0%)	50% (7%) NT	50% (7%) HGF
WBC (/ μ L)	POD1	2100 $\pm$ 1070	1760 $\pm$ 329	3160 $\pm$ 1634
	POD3	8900 $\pm$ 1042	7300 $\pm$ 2935	6040 $\pm$ 2101
	POD7	9000 $\pm$ 2045	12080 $\pm$ 4190	7000 $\pm$ 1994 [†]
Hb (g/dL)	POD1	13.0 $\pm$ 1.0	12.4 $\pm$ 1.0	13.3 $\pm$ 0.6
	POD3	13.8 $\pm$ 0.7	13.5 $\pm$ 0.7	13.4 $\pm$ 0.9
	POD7	12.7 $\pm$ 0.9	11.9 $\pm$ 0.8	13.4 $\pm$ 0.5 ^{††}
Plt (10 ⁴ / μ L)	POD1	53.0 $\pm$ 4.2	38.4 $\pm$ 5.1 ^{***}	45.1 $\pm$ 4.1
	POD3	53.5 $\pm$ 18.7	48.5 $\pm$ 26.3	47.5 $\pm$ 12.9
	POD7	50.3 $\pm$ 11.3	45.5 $\pm$ 10.4	71.5 $\pm$ 2.5 ^{†††}
AST (IU/L)	POD1	978 $\pm$ 171	1921 $\pm$ 929	2110 $\pm$ 905
	POD3	350 $\pm$ 54	379 $\pm$ 287	241 $\pm$ 91
	POD7	262 $\pm$ 114	412 $\pm$ 298	320 $\pm$ 21
T-Bil (mg/dL)	POD1	0.22 $\pm$ 0.04	0.20 $\pm$ 0	0.20 $\pm$ 0
	POD3	0.20 $\pm$ 0	0.66 $\pm$ 1.03	0.20 $\pm$ 0
	POD7	0.20 $\pm$ 0	0.20 $\pm$ 0	0.20 $\pm$ 0
ChE (IU/L)	POD1	65.0 $\pm$ 5.2	56.8 $\pm$ 4.6*	61.2 $\pm$ 4.4
	POD3	63.0 $\pm$ 11.4	60.2 $\pm$ 19.8	63.4 $\pm$ 8.8
	POD7	90.6 $\pm$ 22.5	85.0 $\pm$ 15.8	108.0 $\pm$ 13.0
γ GPT (IU/L)	POD1	2.8 $\pm$ 1.3	2.8 $\pm$ 1.3	3.2 $\pm$ 1.1
	POD3	2.8 $\pm$ 1.3	2.8 $\pm$ 1.3	3.4 $\pm$ 1.1
	POD7	2.8 $\pm$ 1.3	3.6 $\pm$ 2.3	2.0 $\pm$ 0
IL-6 (pg/mL)	POD1	6578 $\pm$ 1702	6321 $\pm$ 1395	
	POD3	4840 $\pm$ 392	5027 $\pm$ 1450	
	POD7	4755 $\pm$ 602	4498 $\pm$ 513	
IFN- γ (pg/mL)	POD1	40785 $\pm$ 6335	42246 $\pm$ 9332	
	POD3	32302 $\pm$ 5673	32464 $\pm$ 10812	
	POD7	32349 $\pm$ 5336	28088 $\pm$ 3436	
TNF- α (pg/mL)	POD1	1466 $\pm$ 285	1461 $\pm$ 372	
	POD3	1265 $\pm$ 122	1195 $\pm$ 479	
	POD7	956 $\pm$ 281	1112 $\pm$ 294	
IL-1a (pg/mL)	POD1	527 $\pm$ 42	465 $\pm$ 139	
	POD3	518 $\pm$ 77	492 $\pm$ 183	
	POD7	560 $\pm$ 38	538 $\pm$ 25	
IL-1b (pg/mL)	POD1	634 $\pm$ 84	577 $\pm$ 162	
	POD3	860 $\pm$ 225	1070 $\pm$ 791	
	POD7	749 $\pm$ 126	1493 $\pm$ 1916	
VEGF (pg/mL)	POD1	1346 $\pm$ 148	1159 $\pm$ 417	
	POD3	1743 $\pm$ 394	1685 $\pm$ 802	
	POD7	1627 $\pm$ 119	1783 $\pm$ 612	

Values are presented as mean $\pm$ standard deviation.

30% (0%) model vs. 50% (7%) NT model: $*p < 0.05$ and $***p < 0.001$.

50% (7%) NT model vs. 50% (7%) HGF model: $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, and $^{\dagger\dagger\dagger}p < 0.001$.

γ GPT: gamma-glutamyl transpeptidase; AST: aspartate aminotransferase; ChE:

cholinesterase; Hb: hemoglobin; HGF: hepatocyte growth factor; IFN- γ : interferon-gamma;

IL-6: interleukin-6; IL-1a: interleukin-1 alpha; IL-1b: interleukin-1 beta; NT: no treatment;

Plt: platelet; POD: postoperative day; T-Bil: total bilirubin; TNF- α : tumor necrosis factor

alpha; VEGF: vascular endothelial growth factor; WBC: white blood cells.

SDC, Materials and Methods

Intraoperative management

Rats were anesthetized with 3.0% isoflurane. They were then subjected to endotracheal intubation (16-G SURFLO intravenous catheter; Terumo, Tokyo, Japan). Artificial respirator (SN-480-7; Shinano, Tokyo, Japan) use was initiated. The respiratory rate was set at 75 breaths per minute during surgery. The isoflurane concentration was adjusted to 1.0% during surgery and 0.5% during the anhepatic period. The surgery was performed under microscopic visualization.

Liver resection techniques

The 30% (7%) model

The abdomen was opened using a midline incision. The caudate, left lateral, left median, and right lobes were ligated with 4-0 silk and resected. When the right lobe was resected, the liver was dissected 2 mm from the inferior vena cava (IVC). The artery and portal vein to the right lobe were individually ligated before dissection to prevent bleeding. The indentation of the right hepatic vein was identified from the anterior aspect of the IVC, and a needle with 6-0 monofilament thread was passed from the indentation to the dorsal surface of the right median lobe and ligated to create the congested area. The abdomen was closed using 4-0 monofilament sutures. During the preliminary experiments, the average percentage of the congested area in the total liver before hepatectomy was 7%.

The 40% (10%) model

The abdomen was opened using a midline incision. The caudate, left lateral, and right lobes were ligated with 4-0 silk and resected. During resection of the left lateral lobe, we ligated the vessels with 7-0 monofilament at the peripheral site of the vessel branching to the left lateral lobe to preserve the artery and portal vein to the left median lobe. Then, the left lateral lobe was ligated with 4-0 silk to preserve the artery and portal vein flow to the left median lobe. The groove with the left median lobe was identified from the dorsal side, and the needle was brought to the surface toward the border between the left and right median lobes and ligated to create the congested area. The abdomen was closed using 4-0 monofilament sutures. Because of anatomical differences, only partially congested area in the left median lobe was occasionally created in some rats; these rats were excluded from this study. During the

preliminary experiment, the average percentage of the congested area in the total liver before hepatectomy was 10%.

Liver transplantation techniques

Donor

The abdomen of donor rat was opened using a midline and transverse incision. Liver resection was performed using the aforementioned procedures. Ligation of the hepatic vein was performed on the back table. The tissue surrounding the liver was dissected, followed by dissection of the bile duct and insertion of a 22-gauge catheter. The branches of the portal vein and gastroduodenal artery were ligated. A 300-U aliquot of heparin was administered through the penile vein, followed by perfusion in the aorta with 50 mL of cold histidine-tryptophan-ketoglutarate (HTK) solution (Custodiol; Dr. Franz Köhler Chemie GmbH, Bensheim, Germany).

Back table

The liver graft was excised and immersed in the HTK solution on ice. A 14-gauge catheter cuff was attached to the portal vein. The venous orifices were identified through the lumen of the IVC, and the right or left hepatic vein was surrounded by 7-0 monofilament using a needle and ligated to create congested area (Supplemental Video). Then, the graft was maintained in the HTK solution at 4 °C for 60 min.

Recipients

The abdomen of the recipient rat was opened using a midline incision. After the removal of the whole liver, the donor graft was placed orthotopically in the abdomen. The suprahepatic IVC was reconstructed using hand-sewn continuous 7-0 monofilament sutures. Portal vein reconstruction was performed using the cuff technique. The median anhepatic time was 12 min. The intrahepatic IVC was reconstructed using hand-sewn continuous 8-0 monofilament sutures. A modified sleeve technique was used to reconstruct the hepatic artery. The proper hepatic artery of the recipient was inserted in the common hepatic artery of the graft, and the stump of the common hepatic artery of the graft was sutured to the tissue around the proper hepatic artery of the recipient using 10-0 monofilament (Supplemental Video). A stent was used to reconstruct the bile duct. Finally, the abdomen was closed with 4-0 monofilament sutures, and the rat was allowed to awaken. The median operative time was 69 min.

To confirm arterial patency, the artery was opened at the time of euthanization on postoperative days 1 and 3, and sufficient blood flow was confirmed. We also included cases without arterial reconstruction to clarify the differences between cases with and without arterial reconstruction in the congested area in the 50% (7%) model ($n = 3$).