A modified kidney-sparing portal vein arterialization model of heterotopic auxiliary liver transplantation increases liver IL-6, TNF-α, and HGF levels and enhances liver regeneration: an animal model

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Research Article

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Abstract

Background and Aim: The implantation site of the donor liver and the vascular reconstruction approach affect the success of partial donor liver transplantation. We investigated the effects of different donor liver implantation sites and vascular reconstruction approaches on liver regeneration using a rat kidney-sparing heterotopic auxiliary liver transplantation model with portal vein arterialization (PVA).

Methods: Sixty male SD rats underwent end-to-end anastomosis of the donor liver portal vein and the right renal artery stent (the control group), or end-to-side anastomosis of the donor liver portal vein and the left common iliac artery (the experimental group).

Results: The experimental group had significantly lower plasma levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, and cholinesterase than the control group (P<0.05). The levels of tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6) and hepatocyte growth factor (HGF) in the liver were significantly higher in the experimental group than that in the control group (P<0.05). Furthermore, H&E staining of the live specimens revealed that the experimental group showed improved hepatocyte regeneration than that of the control group.

Conclusions: The modified kidney-sparing PVA model of heterotopic auxiliary liver transplantation is more conducive to liver regeneration with quicker return of liver function; it may facilitate developing surgical technique to improve liver regeneration in liver transplantation.

Introduction

Liver transplantation has significantly improved the prognosis of patients with acute hepatic failure and is recommended in acute hepatic failure patients who are unlikely to have spontaneous recovery. However, donor liver shortage has severely limited the development of liver transplantation. The liver is an organ with an enormous regeneration potential and acute hepatic failure patients who survive without liver transplant can expect a complete morphological and functional recovery of the liver. Partial donor liver transplant takes advantage of the regenerative ability of the liver to achieve the ultimate goal of a fully regenerated and functioning liver and has become a focus of recent research activities. Donor liver implantation site and vascular reconstruction are particularly important in this regard. For poor portal vein (PV) that cannot be reconstructed and PV thrombosis, Neuhaus *et al*.[1] proposed PV arterialization (PVA) to reuduce the risk of donor liver PV thromobsis. Heterotopic auxiliary liver transplantation with PVA avoids extensive dissection of the native liver and the graft and leave more liver mass for bridging graft and native liver regeneration. Investigators have explored various surgical methods of liver transplantation, aiming at reducing the volume of donor liver needed and solving global donor liver shortage. However, liver transplantation methods are based on the implantation site of the donor liver and PV reconstruction, which determines whether the donor liver could regenerate or not. In the current study, we explored the effects of different donor liver implantation sites and vascular reconstructions in heterotopic auxiliary liver transplantation with PVA on liver regeneration.

Materials And Methods

Animals

Sixty SPF male Sprague-Dawley (SD) rats, weighing 210–260 g each, were obtained from the Laboratory Animal Center, the Third Military Medical University, Chongqing, China. The rats were maintained in a temperature- and humidity-controlled condition with a 12-h light/12-h dark cycle. Rats were allowed free access to standard food and water and underwent an overnight fast before the experiment. The rats were randomly assigned to the control group and the experimental group. The control group underwent end-to-end anastomosis of the donor liver PV and the right renal artery (RA) stent, donor liver suprahepatic caval vein suturing, donor and recipient infrahepatic caval vein end-to-side anastomosis and choledochojejunostomy. The experimental group underwent end-to-side anastomosis of the donor liver PV and the left common iliac artery, reconstruction of the hepatic artery, end-to-side anastomosis of the donor suprahepatic and infrahepatic venal vein and the recipient inferior vena cava (IVC), and choledochojejunostomy.

The study protocol was approved by the ethics committee of the Inner Mongolia Medical University (Permit No.YKD2015102) and the study was carried out in accordance with the institutional and state regulation on the use of experimental animals.

Establishing the PVA model in the experimental group

Donor liver extraction

The schema of heterotopic auxiliary rat liver transplantation with PV arterialization (Fig. 1A). Operative procedures were performed under a microsurgical telescope with 4–6 × magnification. Rats were anesthetized with an isoflurane vaporizer with 5% anesthetic gas to induce anesthesia which was then maintained at 1.5% isoflurane. The rats were placed in the supine position; after depilation and sterilization, heparization was done by injection of 50 U heparin *via* the dorsal vein of the penis. A cruciform incision was made in the upper abdomen and the infrahepatic caval vein was dissected until the left renal vein (RV) was reached without disturbing the liver. The right RV was ligated close to the inferior vena cava (IVC) with a 10 – 0 silk suture. The PV was dissociated until the third tributary was reached. The first PV tributary was dissociated, and the second PV tributary was ligated. A wedge incision 2 mm inferior to the papillary process bile duct was made and the stent was introduced into the common bile duct and a 10/0 double silk suture was used for ligation. The left gastric artery, the splenic artery and the gastroduodenal artery were dissociated from the truncus coeliacus and then ligated and transected; only the common hepatic artery was preserved. The abdominal aorta superior to the truncus coeliacus was dissociated, and a No. 1 stay suture was placed. Then, heparin (500 U) saline solution was injected into the infrahepatic caval vein. A knot above the truncus coeliacus was made with the stay suture, and the abdominal aorta above the knot was clamped and the abdominal aorta below the knot was rapidly punctured, and chilled (4℃) heparin saline solution was continuously infused *via* a micropump at 150 mL/h. The IVC was incised for outflow of the perfusate. The liver was sprinkled continuously with chilled

(4℃) normal saline and the remaining liver was rapidly dissociated. Then, the caudate lobe dorsal to the greater curvature of the stomach was dissociated, and the venous plexus between the left liver and the esophagus and the left triangular ligaments were transected by cauterization. Furthermore, the phrenic vein was ligated close to the liver, and the diaphragm was incised in a circular fashion close to the vena cava foramen. The vena cava was dissociated 2–3 cm into the thoracic cavity and transected. Adhesions between the right lower lobe and the posterior peritoneum were dissociated and transected up to the triangular ligaments. In addition, the venous plexus of the right adrenal gland was dissociated, ligated and transected close to the liver. The infrahepatic caval vein was then transected at the level of the left RV, and the first and second PV tributaries were transected. Meanwhile, the PV was transected at the level of the third PV tributary. Then, an oblique incision was made in the start of the common hepatic artery; after the hepatic artery stent was inserted, the vessel was ligated and fixated, and the common hepatic artery was transected proximal to the ligature and perfusion was discontinued. The donor liver was then removed and placed in a 10-cm dish containing chilled (4℃) heparin saline solution for trimming. Excess fatty tissues in the walls of the suprahepatic and infrahepatic caval vein and PV were removed, and the end for anastomosis was trimmed. The common hepatic artery stent was anastomosed to the first PV tributary, ligated and fixated with 10/0 double silk suture to reconstruct the hepatic artery. Moreover, the contralateral wall of the PV was sutured using 10 − 0 prolene in a running spiral fashion in the proximodistal direction, covering one third of the diameter of the PV. Approximately 70% of the lateral and medial segments of the left lobe were ligated using 9 − 0 silk suture and excised, and the remaining 30% was used as the donor liver and stored at 4℃.

Recipient operation

A median laparotomy was performed. The xiphisternum was pulled cephalically using a large Bulldog clamp, and the intestines were covered with warm wet gauze. A 9 − 0 silk suture was used for ligation and 30% of the left lateral lobe of the recipient liver was excised. The IVC was dissociated from close to the liver to the level of the right RV and also dissociated from below the level of the left RV for 2–3 cm. Given its firm adhesion with the abdominal aorta, the IVC was gently dissociated flush against the abdominal aorta to avoid disrupting its thin wall. The left common iliac artery was identified by moving down the abdominal aorta and dissociated for 1–2 cm. A 9 − 0 silk suture was led out of the two ends of the dissociated vessels for temporarily blocking blood flow. After the donor liver was placed in the right paracolic sulcus, the recipient left common iliac artery was clamped using slip knots with the preset 9 − 0 silk suture and a wedge incision was made in the segment between the two slip knots. End-to-side anastomosis of the donor liver PV and the recipient left common iliac artery (Fig. 1B) was made in a running 10 − 0 prolene suture, and the free flap was embedded in the anastomosis to prevent the blood flow at an excessive speed or in an excessive volume to lessen high perfusion injury in the donor liver. Then, end-to-side anastomosis was made of the donor liver suprahepatic and infrahepatic caval vein and the recipient IVC (Fig. 1C **and D**). The slip knots on the IVC were loosened to allow the blood flow to resume. Then, the slip knots in the left common iliac artery were intermittently loosened. When pulsation of the reconstructed PV and hepatic artery was visible, the anhepatic phase in the donor liver ended; then,

choledochojejunostomy was done. Intra-abdominal irrigation with warm normal saline was done for rapid temperature recovery and postoperative antibiotics was given prophylactically.

Establishing the PVA model in the control group

The surgical steps in extracting the donor liver in the control group were essentially the same as described for the experimental group with the following exceptions. The suprahepatic caval vein was sutured and closed, and the first PV tributary was ligated. In addition, after the right kidney was exposed, the right RA and RV were dissociated at the renal hilum and transected. The right ureter and the right adrenal gland vessels were also ligated. After dissociation of the renal fascia, the right kidney was excised. Then, end-to-end anastomosis was performed of the right RV and the donor liver infrahepatic caval vein using a single running suture. Thereafter, end-to-end anastomosis was undertaken of the right RA and the donor liver PV using the stent method (Fig. 1E). Choledochojejunostomy was then performed. After irrigation with warm normal saline, the abdomen was closed layer by layer.

Postoperative care

All rats received standard postoperative care including ambient warming, electrolyte infusion and high glucose supplementation. Cox-2 inhibitors were used for postoperative pain control. All rats were weighed at postoperative day 1, 3, 5, 7, and 14. The overall condition and food intake were monitored.

Biochemical measurements

Five mL blood was withdrawn *via* the abdominal aorta at postoperative day (POD) 1, 3, 5, 7 and 14 and centrifuged at 3500 rpm/min for 10 min. The plasma contents of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, and cholinesterase were determined.

Histology

Rats were sacrificed at POD 1, 3, 5, 7 and 14 by intraperitoneal injection of barbital. Then, donor liver tissues were obtained and fixed in 10% formaldehyde. The tissue specimens were paraffin embedded and sectioned (5-μm thick). After staining with hematoxylin and eosin (H&E), they were examined under an inverted microscope and photographed.

Immunohistochemistry

Liver tissue sections were dewaxed, hydrated, and washed. After neutralization with 3% endogenous peroxidase and microwave antigen retrieval, slides were preincubated with blocking serum and then incubated overnight with antibodies against TNF-α (ab199013, dilutions 1:200), IL-6 (ab9324, dilutions 1:200), and HGF (ab83760, dilutions 1:200), all from Abcam. Three images of five representative fields were captured under a Canon EOS600D camera connected to a microscope at a magnification of × 200. Images were analyzed with Image-Pro Plus version 6.2 software (Media Cybernetics) using a special function called measurement of integrated absorbance. The integrated absorbance of positive staining of TNF-α, IL-6, and HGF in each photograph was measured and its ratio to total area of each photograph

was calculated as density. The average integrated absorbance value (integrated absorbance/total area) on each slide (three images) was used to represent a particular sample.

Immunoblotting assays

Liver tissues were homogenized and lyzed in RIPA containing PMSF (Beyotine). The cellular lysates were clarified by centrifugation at 12,000 rpm for 15 min. Protein concentration in the lysate was determined using the Bradford method. Immunoblotting assays were performed using a standard protocol. Protein samples were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Thereafter, the proteins were transferred onto PVDF membranes. After blocking in 5% de-fat milk, the membranes were probed with primary antibodies against TNF-α (ab199013, dilutions 1:1000), IL-6 (ab9324, dilutions 1:1000), and HGF (ab83760, dilutions 1:1000) and GADPH (EMM0215, dilutions 1:1000, Elabscience). Protein bands were visualized using an enhanced chemiluminescence method and analyzed using the ImageJ software. Protein expression was normalized against GADPH. The gray ratio of the target bands to that of the internal reference bands was the relative expression of the target proteins.

Statistical analysis

The effect size of rats between the experiment group and the control group was estimated to be at least 0.9. We assumed a statistical power of 0.8 at the confidence level of 0.95. Totally 27 rats in each group were required to arrive at these estimated parameters. Considering about a 10% failure rate of animal experiments, we assigned 30 rats in each group in the study design. Data were expressed as mean ± standard deviation and analyzed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). Student's *t* test was used to compare two groups. $P < 0.05$ was considered statistically significant.

Results

Changes of liver function

Two rats in the control group and 1 rat in the experiment group died due to intraoperative bleeding and were not included. All the other rats resumed activities 1 to 2 h postoperatively and started drinking and eating.

The plasma content of ALT saw a dramatic rise at POD1 in both the control group and the experiment group. The plasma content of ALT in the experiment group rapidly declined from POD 3 and reached the normal range at POD5. Meanwhile, the plasma content of ALT in the control group steadily declined from POD3 but still remained elevated at POD1 4 (Fig. 2A). Similarly, the plasma content of AST declined more steeply in the experiment group than the control group after an initial spike at POD 1 (Fig. 2B). At POD 14, the plasma content of AST returned to normal in the experiment group while remaining elevated in the control group ($P < 0.05$). Total bilirubin also steadily declined and was within the normal range at POD 14 in the experiment group. Meanwhile, total bilirubin remained elevated from POD 1 to 14 in the control

group (Fig. 2C). In addition, serum cholinesterase was significantly higher in the control group than the experimental group at POD 14 ($P < 0.05$) (Fig. 2D).

Histological changes

H&E staining revealed intact lobules, central veins, and portal areas. The central vein was mildly congested, and no apparent infiltration of inflammatory cells was observed. The sinusoidal capillary was congested, and the sinusoidal endothelial cells increased in volume with enlarged nuclei. A small proportion of hepatocytes decreased in volume and pyknosis and necrosis were noted. The portal area showed obvious regenerating hepatocytes with binuclei, portal area, scant inflammatory cells, and mild congestion of interlobular veins (Fig. 3A, B **and C**).

Expression of TNF-α, IL-6 and HGF in liver tissues

We examined the expression of TNF-α in liver tissues by immunohistochemistry and immunoblotting assays. Immunohistochemical staining revealed that TNF-α levels steadily increased in the liver tissues in both the control group and the experimental group from POD 1 and peaked on POD 7 and declined on POD 14. Furthermore, TNF-α levels in the liver tissues of the experimental group were significantly higher than those in the control group at all time points examined ($P < 0.05$) (Fig. 4A). In addition, IL-6 levels in the liver tissues showed similar changes to TNF-α, with a steady increase in both groups from POD 1 and peaked on POD 7 (Fig. 4B). Meanwhile, HGF levels in the liver tissues steadily increased in both the control group and the experimental group from POD 1 and peaked on POD 5; thereafter, HGF levels decreased in both groups. HGF levels in the liver tissues of the experimental group were significantly higher than those in the control group at all time points examined ($P < 0.05$) (Fig. 4C). These findings were confirmed by immunoblotting assays (Fig. 5).

Discussion

The liver has dual blood supply from the PV and the hepatic artery and is the organ with the most abundant blood flow in the body. The PV supplies 70% of the blood to the liver and provides necessary nutrients for maintaining liver regeneratio. Liver transplantation has been increasingly used to treat acute liver failure, fulminant hepatitis, hepatic cirrhosis and metabolic liver diseases. With the rise in the number of patients requiring liver transplanation, donor liver shortage has become an acute issue of global concern and greatly hinders the development of liver transplantation. Cohn[2] and Herrod conceived the idea of liver perfusion using arterial blood instead of blood from the PV in 1952 and showed that normal liver blood flow could be maintained by perfusing the liver with arterial bood with the right flow rate and pressure through the PV, thus promoting liver regeneration and maintaining liver function, showing a novel approach for sovlling the issue of liver transplantation donor liver shortage. Another breakthrough in donor liver transplant site and vascular reocnstruction came from Welch [3] who conceived the idea of auxillary liver transplantation in 1955, which provided a theoretical basis for subsequent studies on liver transplantation and donor liver regeneration. Erhard *et al.* established PVA in heterotopic auxiliary liver

transplantation to reuduce the risk of donor liver PV thromobsis. Margarit *et al.*[4] also used the method to treat fulminant hepatic failurein 2000.

Liver regeneration is a complex process requiring the participiation of multiple cellular factors and fine modulation of HGF; when the liver returns to approxiatmely its normal size, regeneration will cease. Studies have shown that PVA promotes liver regeneration in the initial period but also suport long term growth of the liver[5]. Shimizu *et al.*[6] found that PVA markedly increased oxygen saturation of the perfused liver and greatly reduced consumption of the energy load, ultimately promoting hepatocyte regeneration. Studies have shown that IL-6 is an ipmortant signaling molecule in the initiation of liver regeneration and plays a critical role in initiating the acute phase response in hepatocytes in which multiple types of proteins are rapidly produced to participate in acute or chronic inflammatory repsonse. IL-6, which is produced by both macrophages in the liver and hepatocytes, could activate Kuppfer cells to release TNF-α, which, in turn, stimulate IL-6 production. In the pre-stimulation phase, hepatocyte regeneration is activated and hepatocytes transition from G0 to G1. In the interim, both TNF-α and IL-6 levels rise in the liver tissue and the remaining proteases in the liver are activated. The TNF→TNFR-1→NF-kB→IL-6→STAT3 cascade is activated and initates hepatocyte regeneration, which lasts for 4–6 h post hepatectomy. In addiiton, HGF is an important growth factor for hepatocytes and accelerates cell cycle progression during liver regeneration. HGF is produced by mesenchymal cells in the body and acts on hepatocytes in a paracrine and endocrine manner. HGF is considered an initiator of liver regeneration and is directly mitogentic for hepatocytes. We found that liver regeneration was mainly asosciated with HGF, TNF-α, and IL-6 levels in the liver tissues, and HGF, TNF-α, and IL-6 levels noticealy increased in partially resected donor liver tissues and showed dynamic changes post liver transplant. Improved donor liver implantation site and PVA in the partially resected liver transplant allows proper blood flow at an appropriate rate and pressure in a patent PV, assuring a steady supply of liver regeneration-associated cytokines including TNF-α, IL-6 and HGF, initiating liver regeneration.

Over the recent years, most studies on PVA in heterotopic auxiliary liver transplantation invovle kideny resection and place the donor liver in the kidney-resected area. The PVA model with end-to-end anastomosisof the renal artery and PV unnecessarily sacrifices one kideny and does not follow normal anatomic and physiological relation. In the current study, we estalibshed a novel PVA model by placing the donor liver in the right paracolic sulcus and performing end-to-side anastomosis of the donor liver PV and recipient left common iliac artery. We further investigated the effects on liver regeneration of donor liver placement sites and vascular reconstruction methods for PVA in heterotopic auxiliary liver transplantation in the two models. We found that in our modified PVA model, the volume, velocity, and pressure of blood flow in the donor liver PV was controlled and the donor liver was perfused at an appropriate volume, velocity and pressure, thus lessening the risks of over congestion and rupture of hepatic capsule[7]. As a result, the ahepatic phase was shortened by an abundant supply of oxgenated arterial blood in a short time, thus accelerating liver regeneration. Studes showed that increased volume of blood flow and enhanced oxygen supply after PVA was established was conducive to liver energy metabolism and liver regeneration[8–10]. Other studies further revealed that PVA had no effect on normal liver function but promoted energy metabolism and recovery of hepatic reserve function[11–13]. In our

study, the control group had a higher rate of moratlity than the experimental group; the exact causes are unknown, but could be due to postoperative hemorrage, PV thrombosis, or MODS. The plasma contents of ALT, AST, and total bilirubin declined to normal over time and the amplitude of decrease was significantly greater in the experimental group than the control group.

In the current study, we only used 30% donor liver which greatly reduced the volume of donor liver requried, which could help lay the foundation for solving donor liver shortgage in liver transplantation. Fan *et al.* [14–20] reported that PVA increased oxgyen transport to the donor liver by increasing the extent of liver resection and raising the regeneration of the remnant liver in an animal model. Liver regeneration is mainly initiated by release of cytokines while TNF-α, IL-6 and HGF stimulate hepatocytes to transit from G0 to G1. TNF-α and IL-6 are the main modulators of liver regeneration and induce hepatocyte proliferation by activating NF-kB and STAT3. Lipopolysaccharides, C3a and C5a bind to their respective receptors on macrophages in the liver and play a role in liver regeneration by modulating TNF-α and IL-6. In the partial hepatectomy model, IL-6/sIL-6R in combination with growth factors promote entry of hepatocytes into the cell cycle *via* PI3K/AKT signaling to promote liver regeneration[21]. In addiiton, it was rpeorted that TNF-α and EGF promoted DNA replication during liver regeneration[22]. NK cells and NKT cells upregulate TNF-α and IL-6/STAT3 through immune resposne, and togather with HGF, promote liver regeneration [23]. HGF is an important cytokine in liver regeneration and acctivates *IGFBP* to increase the translation of insulin-like growth factor-binding protein (IGFBP). It has mitogenic activities and hepatoprotective activities. HGF is upregulated during liver regeneration and peaks at day 7 post liver transplant. Hepatocytes are the main effectors of liver regeneration, and the interdependency of cytokiens and growth factors is the main mode of hepatocyte regeneration. Our immunohistochemistry revealed that the expression of TNF-α and IL-6 was markedly increased at POD 1 in both groups and peaked at POD 5 and 7 and thereafter declined, indicating that after partial hepatectomy and PVA, liver regeneration was initated. Notably, the levels of TNF-α and IL-6 in the liver tissues of the experimental group were markedly higehr than those of the control group. In addition, the levels of HGF, a cytokine critical for liver regeneration, showed marked increase in boht groups post transplant but were significantly higher in the experimental group, indicaitng that HGF togather with TNF-α and IL-6 promoted liver regeneration.

In summary, TNF-α, IL-6 and HGF play a critical role in initiating liver regeneration post transplant and TNF-α, IL-6 and HGF levels in the liver tissues could more fully reflect the ability and extent of liver regeneration. The experimental group had significantly higher levels of TNF-α, IL-6 and HGF than the control group, indicating that the liver hemodynmaics of our modified PVA model of heterotopic auxiliary liver transplantation is more conducive liver regeneration. Higher levels of TNF-α, IL-6 and HGF promoted liver regeneration[24, 25]; enhanced liver hemodynamics may improve hepatic access to TNF-α, IL-6 and HGF Therefore, our modified PVA model of heterotopic auxiliary liver transplantation is more conducive to liver regeneration and our findings provide a theoretical basis for future studies on PVA and liver regeneration.

Declarations

Ethics approval and consent to participate: The study protocol was approved by the ethics committee of the Inner Mongolia Medical University (Permit No.YKD2015102) and the study was carried out in accordance with the institutional and state regulation on the use of experimental animals. All methods were carried out in accordance with relevant guidelines and regulations, and the study was carried out in compliance with the ARRIVE guidelines.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: We declare that all the listed authors have participated actively in the study and all meet the requirements of the authorship. Dr. MENG XK designed the study and wrote the protocol, Dr. REN JJ performed research/study,contributed important reagents, Dr. ZHANG JJ managed the literature searches and analyses, undertook the statistical analysis, Dr. LI J wrote the first draft of the manuscript. managed the literature searches and analyses, undertook the statistical analysis, performed research/study,contributed important reagents, and wrote the protocol.

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Figures

Figure 1

(A) The schema of heterotopic auxiliary rat liver transplantation with portal vein arterialization. (B) End-to-side anastomosis of the donor hepatic portal vein and the recipient left common iliac artery is made in a running 10-0 prolene suture, and the free flap is embedded in the anastomosis to prevent the blood flow at an excessive speed or in an excessive volume to lessen high perfusion injury in the donor liver. (C and D) End-to-side anastomosis is made of the donor liver suprahepatic and infrahepatic caval vein and the recipient inferior vena cava. (E) End-to-end anastomosis is undertaken of the right renal artery and the donor liver portal vein using the stent method. (D).

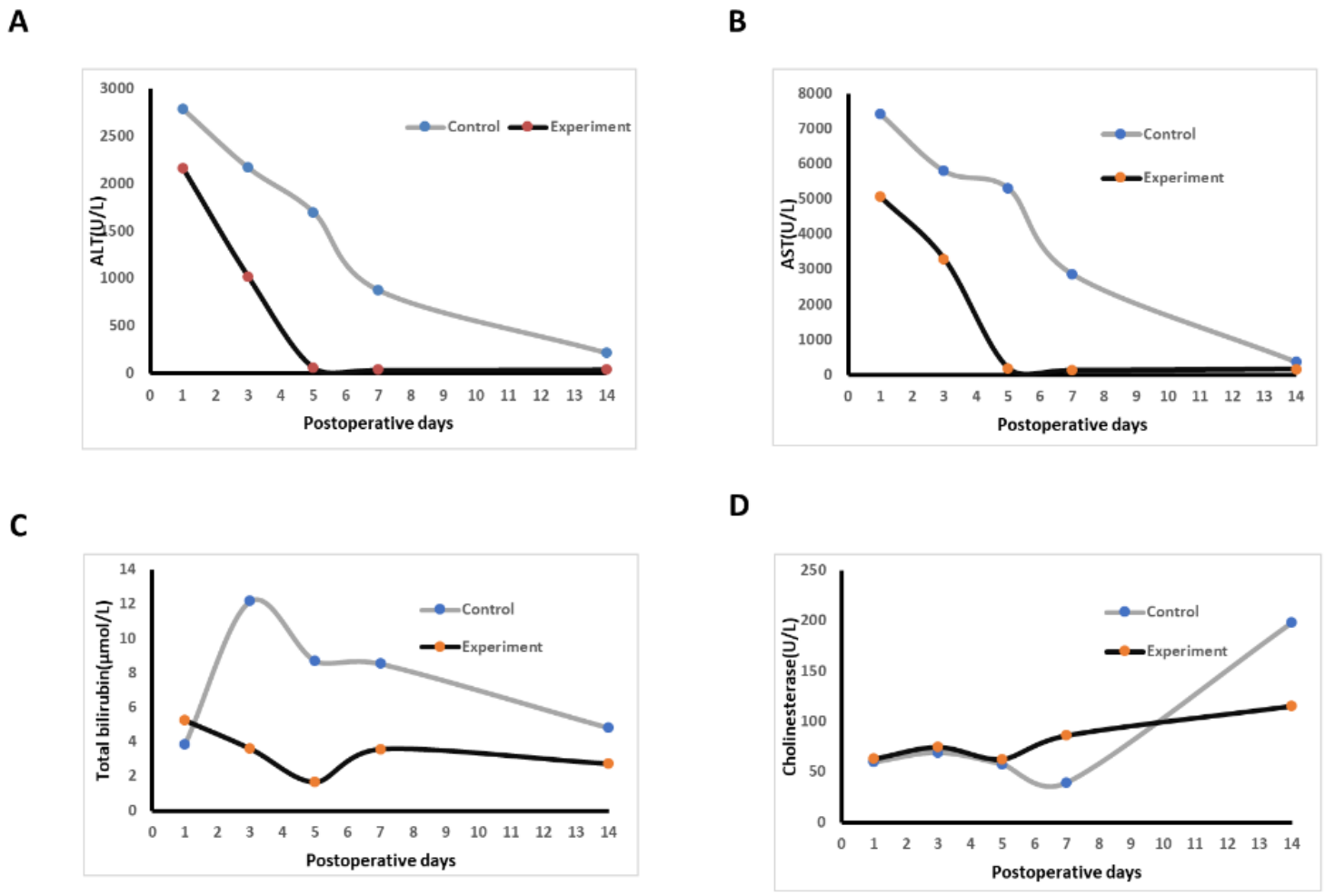

Figure 2

Temporal changes in the plasma contents of liver function parameters post transplant. (A) ALT, (B) AST, (C) total bilirubin and (D) cholinesterase. Data are expressed as mean ± standard deviation. * $P<0.05$ *vs.* the control group.

Figure 3

H&E staining of normal rat liver (A), and donor liver 14 days post transplant in the experimental group (B) and the control group (C). Magnification: 40 x.

Figure 4

Immunohistochemical staining of liver tissues with specific antibodies was undertaken as detailed in Methods. (A) Expression of TNF-α in the liver tissue at post-transplant day 1, 3, 5, 7 and 14 in the experimental group and the control group (left panel). Expression is quantified and shown in bar graphs

(right panel). (B) Expression of IL-6 in the liver tissue at post-transplant day 1, 3, 5, 7 and 14 in the experimental group and the control group (left panel). Expression is quantified and shown in bar graphs (right panel). (C) Expression of HGF in the liver tissue at post-transplant day 1, 3, 5, 7 and 14 in the experimental group and the control group (left panel). Expression is quantified and shown in bar graphs (right panel). Magnification 200 x.

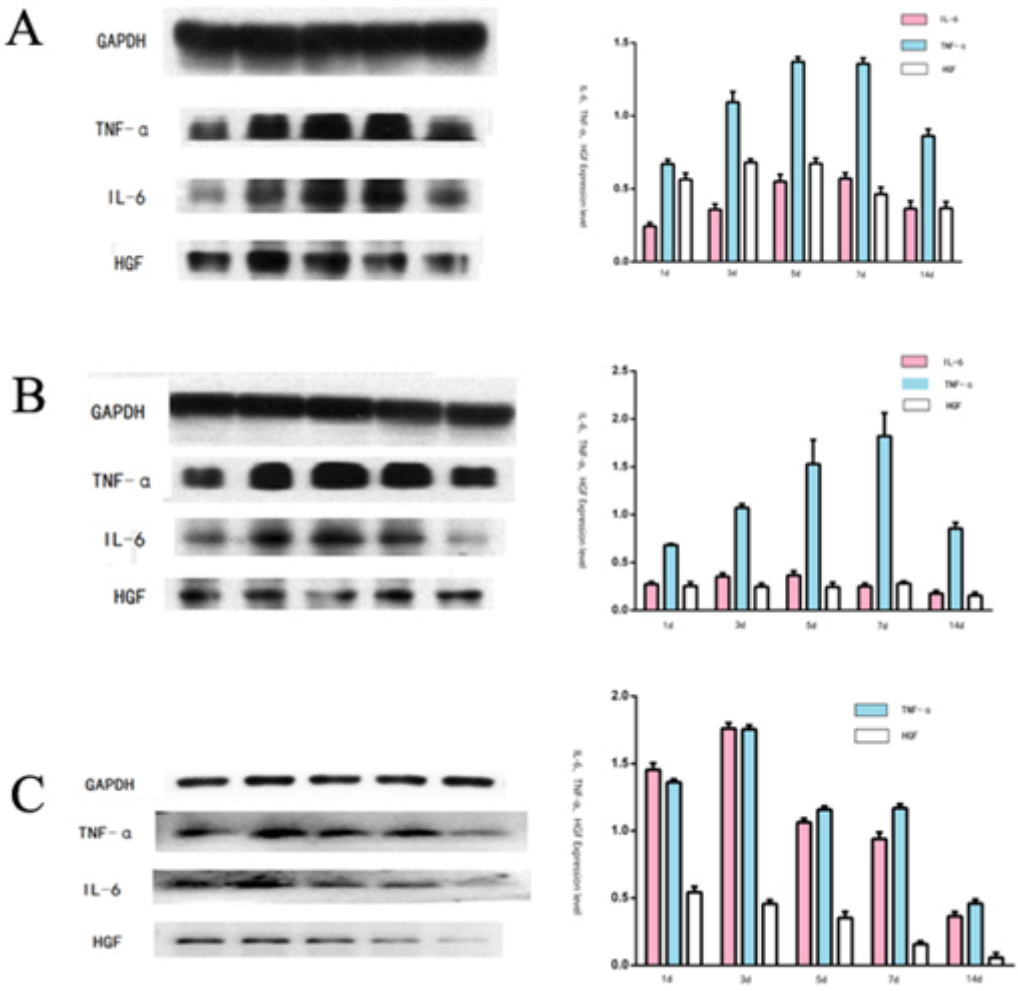

Figure 5

Immunoblotting assays were performed as described in Methods. Expression of TNF-α, IL-6 and HGF in the liver tissue at post-transplant day 1, 3, 5, 7 and 14 in the experimental group (A), the control group (B) and the blank control group (C) (left panel in each). Expression is quantified as described in Methods and shown in bar graphs (right panel in each).