Liver Transplantation in Rats Using Small-for-Size Grafts

A Study of Hemodynamic and Morphological Changes

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Background: Damage to a small-for-size liver graft after reperfusion is frequently observed but the mechanism of injury remains unclear.

Hypothesis: Injury to a small-for-size liver graft is related to the changes of portal pressure and blood flow.

Main Outcome Measures: Survival rates, portal hemodynamics, microcirculatory changes, and morphological changes (by light microscopy and electron microscopy).

Setting: A rat model of nonarterIALIZED orthotopic liver transplantation comparing 2 groups of rats transplanted with whole grafts (100% of recipient liver weight) and small-for-size grafts (30% of recipient liver weight).

Results: Median survival of the rats with small-for-size grafts was 30 hours (range, 27-37 hours). During the first 15 minutes after reperfusion, mean arterial pressure of the small-for-size graft group was significantly lower than that of the whole graft group (10-minute: 100 vs 132 mm Hg, \( P = .04 \); 15-minute: 96 vs 127 mm Hg, \( P = .04 \)). Portal pressure (in centimeters of water) of the small-for-size graft group was significantly higher in the first 20 minutes after reperfusion than the level before the anhepatic phase (5-minute: 15.1 vs 9.3, \( P = .02 \); 10-minute: 16.1 vs 9.3, \( P = .03 \); 15-minute: 13.5 vs 9.3, \( P = .03 \); 20-minute: 13.4 vs 9.3, \( P = .03 \)) and was significantly higher than that of the whole graft group in the first 10 minutes after reperfusion (5-minute: 15.1 vs 9.6, \( P = .02 \); 10-minute: 16.1 vs 10.3, \( P = .04 \)). Hepatic microcirculatory blood flow (in milliliters per minute per 100 g) was also significantly higher in the small-for-size graft group during the first 40 minutes after reperfusion (5-minute: 16.3 vs 9.3, \( P = .02 \); 10-minute: 14.9 vs 6.6, \( P = .02 \); 15-minute: 14.8 vs 5.5, \( P = .02 \); 20-minute: 13.1 vs 7.0, \( P = .02 \); 30-minute: 13.2 vs 8.8, \( P = .04 \); 40-minute: 14.6 vs 7.1, \( P = .02 \)). Light and electron microscopy showed normal morphological features of whole graft up to 24 hours after reperfusion. The small-for-size graft, however, showed sinusoidal congestion, tremendous swelling of mitochondria of hepatocytes, irregular large gap of sinusoidal lining cells, and collapse of the space of Disse.

Conclusions: In a rat model, the portal hemodynamic changes in small-for-size grafts are transient. Progressive damage of the graft may result from microcirculatory failure due to irreversible endothelial injury after reperfusion.


The major concern of adult-to-adult living donor liver transplantation is the adequacy of the size of the graft.1,3 Harvesting a larger graft poses a higher risk for the living donor.4 On the other hand, a small-for-size graft may not only be functionally inadequate for the recipient, but will also sustain injury characterized by cholestasis and histological features of ischemia after implantation.4 The exact mechanism leading to injury of a small-for-size graft after liver transplantation remains unknown. It has been suggested that excessive portal flow secondary to relative portal hypertension may be the cause and that portal decompression may improve graft survival.4

The aim of the present study is to investigate the changes in portal hemodynamics and their correlation with morphological changes of small-for-size grafts on light microscopy and electron microscopy in a rat liver transplantation model.

RESULTS

SURVIVAL RATE

All rats in the whole graft group survived more than 7 days but no rats in the small-for-size graft group survived more than 2 days. The median survival of the small-for-size graft group was 30 hours (range, 27-37 hours).
MATERIALS AND METHODS

ANIMALS

Male Sprague-Dawley rats were used as donors and recipients. Rats were housed in a standard animal laboratory with free activity and access to water and chow. They were kept under constant environmental conditions with a 12-hour light-dark cycle. The rats were fasted 12 hours before operation. All the operations were performed under clean conditions.

EXPERIMENTAL PROCEDURE

The experiment was conducted in 2 groups of rats: whole graft group (n=34) and small-for-size graft group (n=34). A rat model of nonarterialized orthotopic liver transplantation without veno-venous bypass was used. The body weight, graft size, and operative conditions of the groups are shown in the Table. Surgery was performed under pentobarbital anesthesia (intraperitoneal injection, 30-50 mg/kg). The donor’s liver was flushed via the portal vein with 8 mL of cold heparinized saline. In the small-for-size graft group, the lobe ligation technique was used to reduce the graft size on the backtable. The median lobe of the liver was selected to be the graft and the median ratio of the graft weight to recipient liver weight (graft weight ratio) was 28% (range, 21%-33%). The graft was stored in cold saline with a target cold ischemic time of 100 minutes. The suprahepatic vena cava was reconstructed using continuous 7-0 polypropylene sutures. The portal vein was re-anastomosed using the cuff technique modified from Kamada’s method. When the anastomosis of portal vein and suprahepatic vena cava was completed, the liver was reperfused. The anastomosis of infrahepatic vena cava was then completed by the same cuff technique. The common bile duct was connected by telescoping a tube in the donor bile duct into a tube with larger diameter in the recipient. Three milliliters of 0.9% saline and 1 mL of 8.4% sodium bicarbonate was infused via the rat’s penile vein as fluid resuscitation after liver revascularization. After operation, the rats were allowed to recover spontaneously and no further treatment was given.

SURVIVAL STUDY

Ten rats in each group were used for the survival study. Rats that lived more than 7 days after transplantation were considered survivors.

HEMODYNAMIC AND MICROCIRCULATORY STUDY

Six rats in each group were used for hemodynamic and microcirculatory study. After induction of anesthesia, the right jugular vein of the recipient was cannulated with the tip of a catheter positioned at the entrance of the right atrium for monitoring of central venous pressure. The left femoral artery and ileocolic vein were cannulated by a catheter for measurement of the mean arterial pressure and portal pressure, respectively. All catheters were connected via pressure transducers (MLT1030 Blood Pressure; PowerLab System, ADInstruments Pty Ltd, New South Wales, Australia) and Quad Bridge Amp (ML118 Quad Bridge Amp; PowerLab System, ADInstruments Pty Ltd) to a multichannel data recording unit (ML500 PowerLab/800; PowerLab System, ADInstruments Pty Ltd) for continuous pressure monitoring and recording. Microcirculation of the liver graft was measured with laser Doppler BPM Blood Perfusion Monitor, Laserflo BPM; Vasamedics, St Paul, Minn). A laser Doppler probe (model P-440 Solfex Implantable Probe, Laserflo BPM; Vasamedics) was placed on the surface of the median lobe of the graft. Satisfactory contact of the probe and graft surface was maintained during the blood flow detection. Hemodynamic data were analyzed using the PowerLab software system (PowerLab System, ADInstruments Pty Ltd).

BIOCHEMICAL AND MORPHOLOGICAL STUDY

Eighteen rats of each group were used for biochemical and morphological study of graft injury. Liver biopsies were taken on the backtable. Six rats of each group were killed at 30 minutes, 3 hours, and 24 hours after reperfusion for liver biopsy and blood collection. Blood samples (0.5 mL) were collected from the suprahepatic vena cava of the recipients 1 hour and 24 hours after reperfusion for the measurement of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels (Hitachi 747 Automatic Analyzer; Boehringer Mannheim GmbH, Mannheim, Germany).

Liver biopsy specimens were examined under light microscopy with hematoxylin-eosin staining. For electron microscopy examination, the liver biopsy specimens were immediately cut into 1-mm cubes and fixed in 2.5% glutaraldehyde in cacodylate buffer (0.1-mol/L sodium cacodylate-hydrochloride buffer, pH 7.4) overnight at 4°C to 8°C for electron microscopy section. The specimens were examined under a transmission electron microscope (Philips EM208S, Eindhoven, Holland).

STATISTICAL ANALYSIS

Continuous variables were expressed as median and range. The Mann-Whitney U test was used for statistical comparison. Significance was defined as P<.05. Calculations were made with the help of SPSS computer software (SPSS Inc, Chicago, Ill).

HEMODYNAMIC AND MICROCIRCULATORY DATA

During the first 30 minutes after implantation, the mean arterial pressure of the rat in the small-for-size graft group was significantly lower than the basal level. Compared with the whole graft group, the mean arterial pressure was significantly lower at 10 and 15 minutes after reperfusion (Figure 1). The portal pressure of the whole graft group was stable after reperfusion, but there was a significant increase in portal pressure in the small-for-size graft group at 5, 10, 15, and 20 minutes after reperfusion compared with that before transplantation (Figure 2). This increase in portal pressure reached a peak at 10 minutes (increased by 66% above baseline level, 16.1 cm H2O vs 10.7 cm H2O; P=.02) and gradually subsided so that the increase was not significant 30 minutes after reperfusion. Compared with the whole graft group, the portal pressure of the small-for-size graft group was significantly higher at 5 and 10 minutes after reperfusion.
The hepatic microcirculatory flow was significantly higher in the small-for-size graft group than that of the whole graft group during the first 40 minutes after reperfusion (Figure 3). The microcirculatory flow of small-for-size graft group increased by an average of 88% (range, 51%-169%) compared with that of the whole graft group during the first 40 minutes after reperfusion. The central venous pressure was comparable between the 2 groups throughout the first hour after reperfusion (Figure 4).

**Table:**

<table>
<thead>
<tr>
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<th>Whole Graft Group (n = 34)</th>
<th>Small-for-Size Graft Group (n = 34)</th>
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<tbody>
<tr>
<td>Donor body weight, g</td>
<td>281 (246-305)</td>
<td>256 (228-280)</td>
</tr>
<tr>
<td>Recipient body weight, g</td>
<td>289 (269-324)</td>
<td>361 (283-422)</td>
</tr>
<tr>
<td>Graft weight, g</td>
<td>9.0 (8.0-11.8)</td>
<td>3.1 (2.6-4.1)</td>
</tr>
<tr>
<td>Recipient liver weight, g</td>
<td>10 (8.5-11.7)</td>
<td>11 (8.6-12.8)</td>
</tr>
<tr>
<td>Graft weight ratio, † %</td>
<td>94 (85-111)</td>
<td>28 (21-35)</td>
</tr>
<tr>
<td>Cold ischemic time, min</td>
<td>94 (80-118)</td>
<td>106 (92-120)</td>
</tr>
<tr>
<td>Anhepatic phase, min</td>
<td>20 (19-22)</td>
<td>18 (17-20)</td>
</tr>
</tbody>
</table>

*Data are expressed as median (range).
†Graft weight ratio = graft weight/recipient liver weight.

**Figure 1.** Time course of mean arterial pressure. Base indicates after induction of anesthesia; before, before removal of recipient liver; asterisk, P < .05 compared with basal level of mean arterial pressure; and dagger, P < .05 compared with whole graft group.

**Figure 2.** Time course of portal pressure. Base indicates after induction of anesthesia; before, before removal of recipient liver; asterisk, P < .05 compared with portal pressure before removal of recipient liver; and dagger, P < .05 compared with whole graft group.

**Figure 3.** Time course of hepatic microcirculatory flow after reperfusion. Asterisk indicates P < .05 compared with whole graft group.

**Figure 4.** Time course of central venous pressure. Base indicates after induction of anesthesia; before, before removal of recipient liver.

**Figure 5.** Serum alanine aminotransferase (ALT) (A) and aspartate aminotransferase (AST) (B) levels 1 hour and 24 hours after reperfusion. Asterisk indicates P < .05 24 hours vs 1 hour after reperfusion in the small-for-size graft group; dagger, P < .05 small-for-size grafts vs whole grafts 24 hours after reperfusion.

**BIOCHEMICAL DATA**

At 1 hour after reperfusion, there was no significant difference in the serum ALT and AST levels between the 2 groups (Figure 5). The enzyme levels increased significantly at 24 hours after reperfusion in the small-for-size group (ALT: 1013 vs 357 U/L, P = .02; AST: 879 vs 357 U/L).
U/L, \( P = .04 \), while there was no significant change in the whole graft group (ALT: 486 vs 414 U/L, \( P = .87 \); AST: 414 vs 517 U/L, \( P = .61 \)). Serum ALT and AST levels at 24 hours after reperfusion were significantly higher in the small-for-size graft group than those of the whole graft group (Figure 5).

MORPHOLOGICAL EXAMINATION

Light Microscopy

In the whole graft group, light microscopy showed normal morphological changes of the graft after 100 minutes of cold preservation. Thirty minutes, 3 hours and 24 hours after reperfusion, the hepatic lobular architecture was preserved. The hepatocytes and portal tracts showed normal morphological changes.

In the small-for-size graft group, there was severe sinusoidal congestion and vacuolar change in the cytoplasm of hepatocytes 30 minutes after reperfusion (Figure 6A). At 3 hours after reperfusion, sinusoidal congestion and vacuolar change in cytoplasm of hepatocytes were persistent. After 24 hours of reperfusion, apoptosis was seen in the hepatocytes (Figure 6B).

Electron Microscopy

Hepatocytes and sinusoidal cells had normal appearance under transmission electron microscopy examination before implantation and at 30 minutes, 3 hours, and 24 hours after reperfusion in the rats implanted with whole graft (Figure 7A). Chromatin in the nucleus appeared normal. Mitochondria were elliptical, with well-visualized cristae. The endoplasmic reticulum was intact, and there were numerous microvilli in the space of Disse. The sinusoidal lining cells were intact.

In the small-for-size graft group, mitochondrial swelling and vacuolar changes in cytoplasm of hepatocytes were present at 30 minutes and 3 hours after reperfusion. He-
patent belling, sinusoidal congestion, and irregular large gap of sinusoidal lining cells were also found. Twenty-four hours after reperfusion, collapse of the Disse space was obvious and the integrity of endothelial cells was disrupted (Figure 7B).

COMMENT

Both animal experiments and clinical experiences have shown lower survival rate after liver transplantation in recipients with small-for-size grafts.1-3,5,7 The minimum ratio of the donor and recipient in the rat and canine liver transplant model were 53% and 25%, respectively.5,7 The safety limit in clinical practice was 40% of the ideal liver weight or 1% of recipient body weight.1,2 Both reduced metabolic and synthetic capacity as well as enhanced liver parenchymal injury might be responsible for the lower graft survival.3 The mechanism of injury may be comparable to the one that resulted in progressive necrosis of the liver remnant developed after major hepatectomy in a rat model.8 It was postulated that the excessive portal blood flow relative to a small-for-size graft would induce portal hypertension after reperfusion, which in turn leads to injury of the graft. In a model of canine quarter liver transplantation,7 portal pressure was markedly elevated after reperfusion of graft and portal decompression by portal-systemic shunt improved the survival. Though effective, a portal-systemic shunt may result in long-term “portal steal” and adversely affect the regeneration of the liver graft. Therefore, a detailed elucidation of the portal hemodynamic changes and the mechanism of injury of small-for-size grafts is necessary and will provide helpful information for transplant surgeons to develop new strategies to minimize the injury and to improve the prognosis of liver transplantation using small-for-size grafts.

To elucidate the possible mechanism of small-for-size graft injury, continuous monitoring and recording of the various hemodynamic parameters were performed in the present study. The PowerLab monitoring system, comprising PowerLab hardware and software, records and displays experimental data on the screen in real time. It is widely used in animal and human hemodynamic research including blood pressure and blood flow.9-11 The laser Doppler flowmetry is a noninvasive technique that allows continuous evaluation of microvascular perfusion even after ligature of the hepatic artery.12-14 Combined with PowerLab software under Windows 98, hemodynamic data could be analyzed systematically and accurately.

The present study showed that the significant changes in hemodynamics and hepatic microcirculation in rats receiving small-for-size grafts were transient and mainly occurred in the acute phase after reperfusion. Since the maximum additional hepatic blood flow volume that the liver can tolerate is 20%,15 an 88% increase of the blood flow in the small-for-size graft during the first 40 minutes after reperfusion is obviously harmful to the graft. However, the portal hypertension related to small-for-size graft subsided within 30 minutes after reperfusion. The resolution of the transient portal hypertension might be due to the reduction in the systemic blood pressure, which was related to poor graft function.

The sinusoids are the principal vessels involved in the transvascular exchange between blood and the parenchymal cell. The hepatic sinusoidal cells play a critical role in the maintenance of hepatocyte function, under both physiological and pathological circumstances. The homeostasis of hepatic microcirculatory environment is crucial for the early recovery of graft function after reperfusion.10-18 The disruption of sinusoidal lining cells and sinusoidal congestion were compatible with sinusoidal injury due to the transient portal hypertension and excessive portal blood flow relative to small-for-size graft. The resulting derangement in hepatic microcirculation was progressive and was reflected by severe sinusoidal stasis and collapse of the Disse space after 24 hours of reperfusion,8,10-20 even though the hemodynamic changes were transient. The formation of blebs and severe swelling of mitochondria in hepatocytes were due to ischemia secondary to the sinusoidal injury.16 Liver cell injury as reflected by dramatic elevation of ALT and AST at 24 hours after reperfusion was observed, which correlated with the tremendous swelling of mitochondria of hepatocytes and the occurrence of apoptosis. It should be noted that the present study was conducted in rats without preexisting portal hypertension. In case of cirrhosis, we suspect that the degree of injury to small-for-size liver grafts may be even worse and that the portal hemodynamic changes may be different.

In summary, these results suggest that transient portal hypertension and excessive hepatic blood flow were found in the first 30 minutes after reperfusion of small-for-size liver grafts in a rat model. Although the portal hemodynamic changes were transient, the small-for-size graft injury reflected by biochemical and morphological changes was continuous and progressive, suggesting that irreversible sinusoidal damages have occurred in the early phase after reperfusion.

In conclusion, in a rat model, portal hemodynamic changes in small-for-size grafts are transient. Progressive damage of the graft may result from microcirculatory failure due to irreversible endothelial injury after reperfusion.

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