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# Sinusoidal microcirculatory changes after small-for-size liver transplantation in rats

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**Keywords**
liver transplantation, portal hypertension, sinusoidal microcirculation small-for-size graft.

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## Summary

Small-for-size graft injury is characterized by portal venous hypertension and loss of intracellular homeostasis early after transplant. The long-term alteration of sinusoidal microcirculatory hemodynamic state remains unknown. A syngeneic rat orthotopic liver transplantation model was developed using small-for-size grafts (35% of recipient liver weight) or whole grafts (100% of recipient liver weight). Graft survival, portal pressure, liver function, hepatocellular apoptosis as well as morphological changes (by light microscopy and electron microscopy) were assessed. Sinusoidal microcirculatory hemodynamics was examined by intravital fluorescence microscopy. Although portal hypertension lasted only for 1 h after performance of small-for-size liver transplantation, a sustained microcirculatory disturbance was accompanied by dramatic reduction of sinusoidal perfusion rate, elevation of sinusoidal diameter as well as increase in the number of apoptotic hepatocytes during the first 7 days. These resulted in lower survival rate (50% vs. 100%, $P = 0.012$), higher level of liver function, and more severe morphological changes, which could induce small-for-size syndrome. In conclusion, persistent microcirculatory hemodynamic derangement during the first 7 days after reperfusion as well as transient portal hypertension is significant manifestation after small-for-size liver transplantation. Long-term microcirculation disturbance displayed as decrease of sinusoidal reperfusion area and increase of spread in functional liver mass seems to be the key factor for graft injuries.

## Introduction

Since the first successful living donor liver transplantation (LDLT) in a child patient [1] and an adult patient [2], it has become the established method to reduce the number of patients on the waiting list and is considered as an alternative to standard liver transplantation. Although the number of LDLTs has increased rapidly in recent years, ability to meet the demand for the procedure substantially is severely limited by liver tissue availability including optimal-size graft. Many researchers have reported that small-for-size liver grafts (graft-to-recipient weight ratio of <0.8–1% or graft volume/standard liver volume of <30–40%) decreases recipient survival and adversely affects graft function after transplantation [3–6]. In addition, the severity of the recipient's illness also affects the outcomes of transplantation, in which recipients with Child's class B and C had poorer prognosis. In these cases, even a patient who receives a reasonable graft from a living donor will develop severe complications which trigger a pathophysiologic cascade that clinically manifests as portal hypertension, ascites, coagulopathy, and hyperbilirubinemia, collectively termed small-for-size syndrome (SFSS) [7–9] and resulting in graft failure.

The mechanisms of small-for-size graft failure have been studied in recent years and the portal venous

 

hypertension is considered to be one of the most important factors [8,10–14] leading to early allograft dysfunction in small-for-size liver transplantation. It can induce mechanical injury to the sinusoidal structure and trigger a series of graft injuries such as: (i) up-regulation of proinflamatory cytokines such as early growth response factor-1, heat shock protein-70[14,15]; (ii) abnormal expression of the mitogen-activated protein kinase signaling pathway-associated protein [16]; and (iii) an imbalance of vasoactive-related molecules such as endothelin-1 (ET-1) heme oxygenase-1, and nitric oxide [10,14,17–19]. To date, studies have focused on graft impairment during the first 24 h after reperfusion and little data on long-term graft alteration has been reported. Although many studies presumed microcirculation deterioration after small-for-size liver transplantation, the microcirculatory hemodynamic states with different sizes of graft had rarely been studied, especially in the later phase after reperfusion. We hypothesized that excessive portal flow could be attributed to postoperative small-for-size liver dysfunction caused by persistent sinusoidal microcirculatory injury even though the graft survived for the first day after reperfusion.

Intravital fluorescence microscopy (IFM) allows for direct visualization of dynamic changes in functional microcirculatory changes, and has been shown to be useful in many conditions including hepatic ischemia/reperfusion injury (IRI), liver regeneration and other physiological and pathological processes [20–23]. In this experiment, a rat model of syngeneic liver transplantation with small-for-size and whole grafts was designed to examine sinusoidal microcirculatory states by IFM in either early or later time period after reperfusion. Graft injuries were also detected by conventional measurements.

## Materials and methods

### Animals

Male inbred Lewis rats (weighing 200–280 g) obtained from Beijing Laboratory Animal Research Center (Vital River Laboratory Animal Technology Co., Ltd, Beijing, China) were used as donors and recipients. The study protocol conformed to the Guidelines for the Care and Use of Laboratory Animals as adopted and promulgated by the United States National Institutes of Health and was approved by the Animal Ethics Review Committees of Zhejiang University.

### Experimental design and surgical procedure

The animals were divided into two groups according to the ratio of graft weight to recipient liver weight: the whole graft liver transplantation group (WLT group) ($n = 48$), and the small-for-size graft liver transplantation group (SLT group) ($n = 48$) the surgical parameters are listed in (Table 1). Another five rats were chosen for baseline value.

Surgery was performed under sodium pentobarbital anesthesia (intraperitoneal injection, 30–50 mg/kg). The lobe ligation technique was used to reduce the graft size on the back table, and the reduced size grafts were immediately weighed as previously described [14,16]. The median lobe of the liver was selected to obtain 35% small-for-size liver graft. The liver grafts were preserved in ice-cold Ringer's lactate solution for 30 min before implantation into the recipients. Orthotopic partial liver syngenic graft transplantations were performed with the two-cuff technique, which was first established by Kamada. After operation, rats were allowed to spontaneously recover, and no further treatment was given. More than 90% of the rats survived this surgery. Recipients were examined by IFM, and were sacrificed at 1 h, 1 , 3 , and 7 days after transplantation $n = 5$ each) to collect graft liver and blood specimens. Recipients were also sacrificed at 3 and 6 h after transplantation ($n = 5$ each) to harvest comparative specimens. Eight recipients per group were used for portal pressure measurement. Ten recipients per group were allowed to survive until they died and 7-day survival rate was studied.

### Survival study

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

### Portal pressure measurement

After induction of anesthesia, the ileocolic veins were cannulated by a catheter for measurement of portal pressure. The catheter was connected via the pressure transducer (YPJ01 Pressure Transducer, physiological experiment system, Chengdu Instruments, Sichuan, China) to a multichannel data-recording unit (RM6240C, physiological experiment system, Chengdu Instruments) for continuous pressure monitoring and recording. Portal pressure fluctuation in recipients was recorded before removing the liver (as the basal level), at the time of clamping, and after reperfusion. All data were analysed using the physiological experiment software system (RM6240 physiological experiment system, Chengdu Instruments).

### Intravital fluorescence microscopy

Rats were anesthetized using sodium pentobarbital (intraperitoneal injection, 30–50 mg/kg), and were placed

in prone position on a heating pad for maintenance of body temperature. The median lobe was exteriorized on a plasticine disc allowing an ideal placement. The exposed area was immediately covered with a glass slide to prevent tissue drying and influence of ambient oxygen.

Using an inverted fluorescence Olympus IX81 microscope (Olympus Optical, Tokyo, Japan) equipped with a 100V mercury lamp (X-cite 120; Olympus Optical), different filter sets for blue (excitation/emission wavelength = 450–490 nm/>520 nm), green (530–560 nm/>580 nm) and ultraviolet (330–380 nm/>415 nm) light epi-illumination and a dichroic mirror controlled by an Olympus IX2-UCB controller, images were taken by a CCD video camera (DP 30BW; Olympus Optical) and transferred to a video system for offline analysis. With the use of a water immersion objective (×4/0.13; ×10/0.30; ×20/0.50 W; Olympus Optical), total optical magnifications of ×64, ×160 and ×320 were achieved on the video screen.

Liver microcirculation was quantitatively analysed after tissue contrast enhancement by dextran-fluorescein (MW 40 000; 2 μmol/kg i.v.; D1844; Invitrogen, Molecular Probes, Inc., Eugene, OR, USA) using blue light epi-illumination. For analysis of hepatocytes and apoptotic cell death, *in vivo* staining of hepatocellular nuclei was achieved by intravenous injection of bisbenzimide (Hoechst 33342; 10 μmol/kg; 620-050-M050; Alexis Biochemicals, San Diego, CA, USA) and ultraviolet epi-illumination. Ten to 15 different nonadjacent hepatic acini were recorded for each rat.

### Analysis of microcirculation

The recorded images were digitized and analysed frame-to-frame by a computer-aided image analysis software system (Image-Pro Plus 6.0; Media Cybernetics, Sliver Spring, MD, USA). The following cellular and microcirculatory parameters were evaluated: (i) sinusoidal perfusion (%), presented as the percentage of perfused sinusoids in relation to all visible sinusoids; (ii) sinusoidal density (cm/cm$^2$), given as the total length of sinusoids per observation field, regardless of perfusion; (iii) sinusoidal diameter (μm), given as the average internal diameter of 10 sinusoids at the mid-zonal regions of each observation area; (iv) red blood cell (RBC) velocity (μm/s) in 10 individual sinusoids per observation area using the track-object method (Image-Pro Plus); (v) the distance between two hepatocellular nuclei (μm) along hepatocellular columns; and (vi) apoptotic cells (n/mm$^2$), given as the number of cells which showed apoptosis-associated condensation and crescent-shaped formation of chromatin per observation field.

**Table 1.** Surgical parameters in each group.

| Surgical parameters | SLT group ($n$ = 48) | WLT group ($n$ = 48) |
|---|---|---|
| Graft ratio (%) | 36.2 ± 3.4* | 97.0 ± 5.24 |
| Time for donor (min) | 25.3 ± 4.7* | 14.4 ± 2.5 |
| Time for ischemia (min) | 46.6 ± 2.2 | 43.7 ± 1.5 |
| Recipient weight (g) | 275.5 ± 14.9 | 269.4 ± 10.4 |
| Anhepatic phase (min) | 13.2 ± 1.5 | 12.9 ± 1.6 |
| Time for recipient (min) | 43.4 ± 2.8 | 41.3 ± 3.5 |

WLT, whole graft liver transplantation; SLT, small-for-size liver transplantation.
*$P$ < 0.05 vs. WLT group.

### Serum biochemical examination

All blood samples were centrifuged at 2000 *g* for 10 min at 4 °C, and the serum was frozen at −80 °C for further measurement. Serum biochemical parameters were measured using an auto-analyzer (Hitachi 7600; Hitachi Co., Ltd., Tokyo, Japan).

### Morphological studies

Liver tissue were fixed in 10% formaldehyde at 4 °C overnight and embedded in paraffin. Sections at 4-μm thickness were prepared and processed with hematoxylin and eosin staining and the morphometrics detection were performed using light microscopy. The specimens for electron microscopy were immediately cut into 1-mm cubes and fixed in 2.5% glutaraldehyde in phosphate buffer solution at 4 °C overnight. The sections were examined under a transmission electron microscope (JEM-1230, JEOL Ltd., Tokyo, Japan).

### Statistical analysis

Data are expressed as mean ± standard deviation, and statistical analyses were performed with SPSS 13.0 software (SPSS, Inc., Chicago, IL, USA). One-way analyses of variance (ANOVA), followed by Student–Newman–Keuls (SNK) *post hoc* tests were used for statistical comparison. Survival rates were assessed by the Kaplan–Meier method. The log-rank test was used to compare significance. Significance was defined as $P < 0.05$.

## Results

### Lower 7-day survival rate

Seven-day graft survival rates were 50% (5/10) in the SLT group and 100% (10/10) in the WLT group ($P = 0.012$; Fig. 1). However, among the five recipients in the small-for-size (SFS) group which survived 7 days, two rats

| Group | Survival time (days) |
|---|---|
| WLT | 1,2,3,5,5,13,15,21,>30,>30 |
| SLT | 10,14,25,>30,>30,>30,>30,>30,>30,>30 |

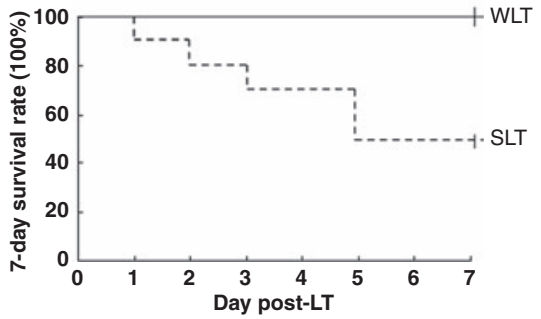


**Figure 1** Survival time and 7-day survival rate after transplantation with whole and small-for-size liver grafts ($n$ = 10). Survival in WLT group (solid line) by day 7 was 100%, which dropped to 50% in SLT group (dash line), respectively ($P$ = 0.012). WLT, whole graft liver transplantation; SLT, small-for-size liver transplantation.

lived longer than 1 month, and others died at 13, 15, and 21 days ($n$ = 1 for each day) after transplantation.

### Elevation of serum biochemical parameters

Both serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were significantly increased, and peak levels occurred at 6 h in the WLT group (ALT: 1825.2 ± 249.3 U/l vs. 994.4 ± 357.2 U/l, $P < 0.05$; AST: 1560.2 ± 586.3 U/l vs. 1159.4 ± 540.7 U/l, $P < 0.05$; Fig. 2). The peak levels of serum ALT and AST in the SLT group occurred at day 1, and were significantly higher than those in the WLT group at the same time (ALT: 1507.2 ± 422.3 U/l vs. 888.8 ± 185.3 U/l, $P < 0.05$; AST: 1798.6 ± 445.0 U/l vs. 1006.2 ± 330.7 U/l, $P < 0.05$; Fig. 2). Although decreased in both groups after day 1, the serum enzyme level in the SFS group was still higher than the same in the WLT group. The serum level of total bilirubin (TB) increased significantly from day 3 after operation in the SLT group (day 3: 19.0 ± 16.3 μmol/l vs. 1.6 ± 0.5 μmol/l, $P < 0.05$; day 7: 38 ± 12.5 μmol/l vs. 3.0 ± 1.6 μmol/l $P < 0.05$; Fig. 2).

### Transient portal hypertension at very early phase of reperfusion

There was no significant difference in portal pressure between the two groups before the removal of the liver or after portal vein clamping. Portal pressures after reperfusion in the SLT group were significantly higher than the same 1 h before reperfusion in relation to those in the WLT group (Fig. 3). There was no statistical difference in portal pressure 1 h after reperfusion.

### Disturbed hepatic microcirculation

Sinusoidal RBC velocity dramatically increased after reperfusion and maintained at a higher level in the SLT

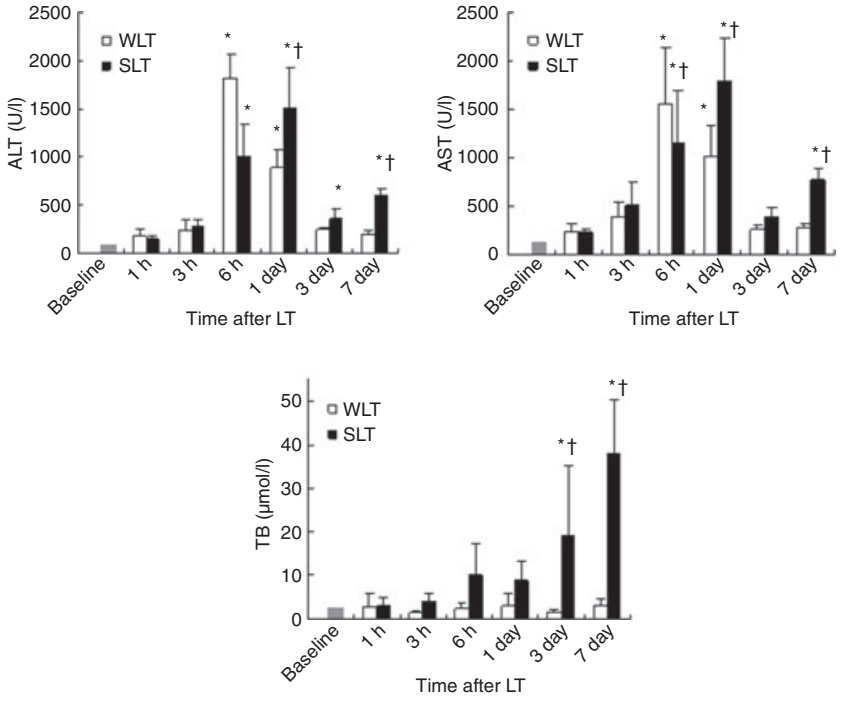


**Figure 2** Serum levels liver function (ALT, AST and TB) in after transplantation with different size of grafts ($n$ = 5). WLT, whole graft liver transplantation; SLT, small-for-size liver transplantation; *$P < 0.05$, vs. baseline; †$P < 0.05$, SLT group vs. WLT group at respective time point.

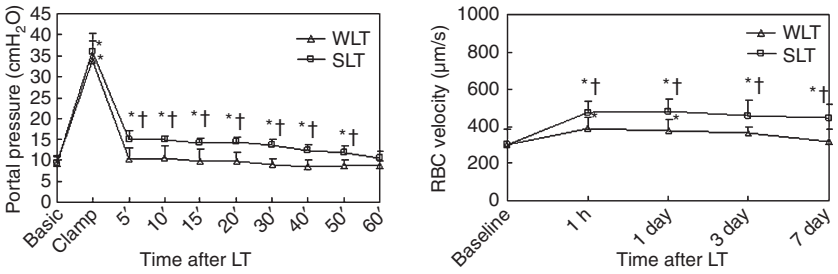


**Figure 3** Portal pressure and sinusoidal RBC velocity ($n$ = 5) after transplantation. Transient portal hypertension was found in the SLT group ($n$ = 8) while no increase of portal pressure occurred in the WLT group ($n$ = 8). Sinusoidal velocity was maintained at a significantly high level during 7 days after transplantation in the SLT group ($n$ = 5) when compared with WLT group. WLT, whole graft liver transplantation; SLT, small-for-size liver transplantation; *$P$ < 0.05, vs. baseline; †$P$ < 0.05, SLT group vs. WLT group at respective time point.

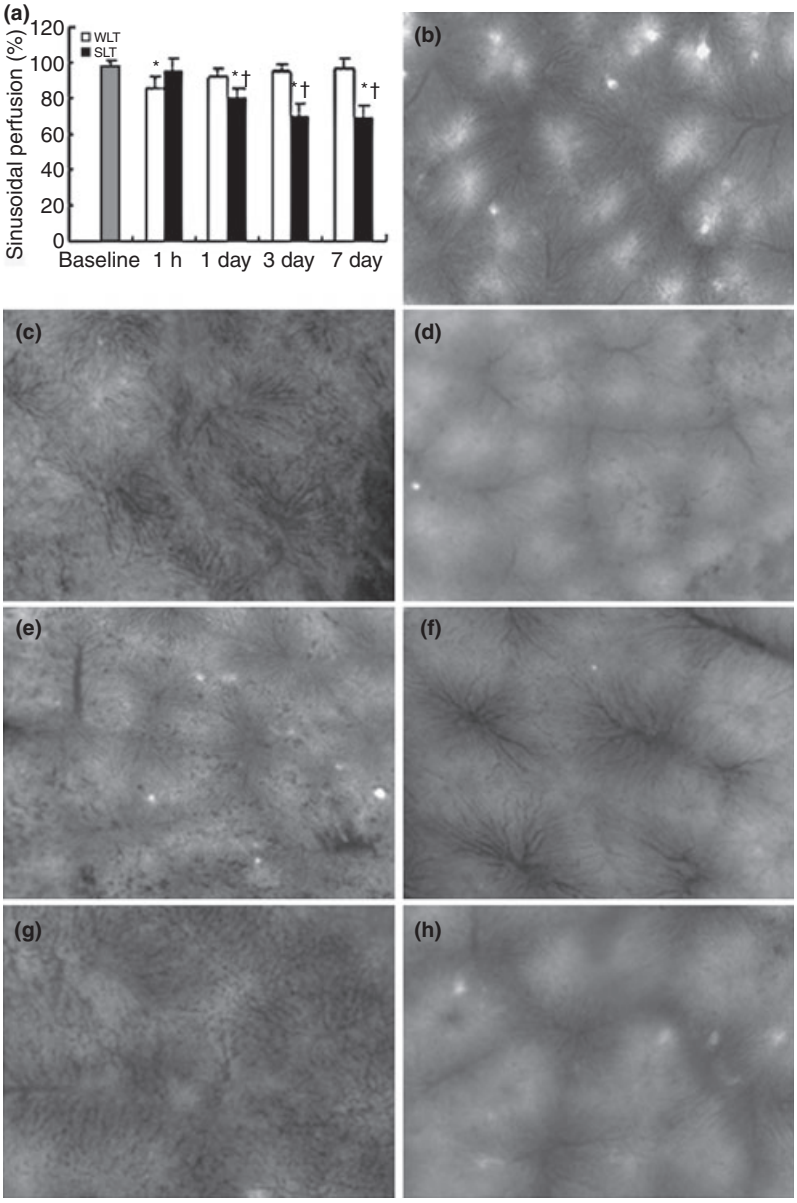


**Figure 4** Sinusoidal perfusion after transplantation with whole and small-for-size liver grafts ($n$ = 5).
(a) Quantitative analysis of sinusoidal perfusion rate (%); WLT, whole graft liver transplantation; SLT, small-for-size liver transplantation; *$P$ < 0.05, vs. baseline; †$P$ < 0.05, SLT group vs. WLT group at respective time point.
(b) Representative intravital fluorescence microscopic images of sinusoidal perfusion in normal liver; ×64. (c, e and g) Representative intravital fluorescence microscopic images of sinusoidal perfusion in SLT group at day 1, 3 and 7 respectively. (d, f and h) Representative intravital fluorescence microscopic images of sinusoidal perfusion in WLT group at day 1, 3 and 7 respectively. Original magnification ×64.

group compared to WLT group (1 h: 472.7 ± 63.4 μm/s vs. 384.9 ± 65.3 μm/s, $P < 0.05$;day 1: 481.1 ± 70.3 μm/s vs. 376.4 ± 62.4 μm/s, $P < 0.05$; day 3: 454.3 ± 90.6 μm/s vs. 367.0 ± 34.2 μm/s, $P < 0.05$; day 7: 443.9 ± 76.1 μm/s vs. 316.3 ± 69.1 μm/s, $P < 0.05$; Fig. 3). As a consequence of a hepatic hyper-hemodynamic state, microcirculatory injuries in the SLT group presented as significantly lower sinusoidal perfusion rate (day 1: 80.4 ± 5.3% vs. 92.1 ± 4.5%, $P < 0.05$; day 3: 70.1 ± 6.6% vs. 95.4 ± 3.7%, $P < 0.05$; day 7: 67.5 ± 8.2% vs. 97.8 ± 4.6%, $P < 0.05$; Fig. 4) as well as larger sinusoidal diameter (1-h: 9.2 ± 1.3 μm vs. 6.6 ± 1.3 μm; day 1: 8.4 ± 1.0 vs. 7.0 ± 0.9 μm; day 3: 9.1 ± 1.3 μm vs. 7.8 ± 0.7 μm; day 7: 9.2 ± 1.1 μm vs. 8.1 ± 0.9 μm; $P < 0.05$; Fig. 5a and e upper line) and smaller sinusoidal density ($P < 0.05$ versus both normal and WLT groups; Fig. 5b). However, RBC velocity in the WLT group decreased only slightly during the first day and gradually increased thereafter. Accordingly, sinusoidal perfusion and diameter changed transiently and returned to the baseline. Hence, the alteration under IFM was more severe in the SLT group.

### Increase of hepatocyte apoptosis

*In vivo* analysis of bisbenzimide-stained hepatocytes revealed an increased number of apoptotic hepatocytes per $mm^2$ area in the SLT group compared to WLT group (day 3: 5.2 ± 1.5 n/$mm^2$ vs. 3.1 ± 1.0 n/$mm^2$, $P < 0.05$; day 7: 12.4 ± 3.0 n/$mm^2$ vs. 3.1 ± 1.2 n/$mm^2$, $P < 0.05$; Fig. 5d and e lower line). Moreover, the nucleo-nuclear distance of hepatocytes along the individual columns increased significantly in the SLT group (day 1: 17.5 ± 1.4 μm vs. 16.6 ± 1.0 μm, $P < 0.05$; day 3: 18.1 ± 1.1 μm vs. 16.4 ± 0.9 μm, $P < 0.05$; day 7: 18.3 ± 1.2 μm vs. 16.5 ± 1.0 μm, $P < 0.05$; Fig. 5c), which suggested hepatocellular swelling.

### Irreversible morphological alteration

In SLT group, graft liver showed apparent sinusoidal congestion and hepatocellular degeneration accompanied by scattered necrotic and apoptotic cell during the 24-h period after reperfusion. SFS graft congestion was manifested as focal hemorrhage into the portal tract connective

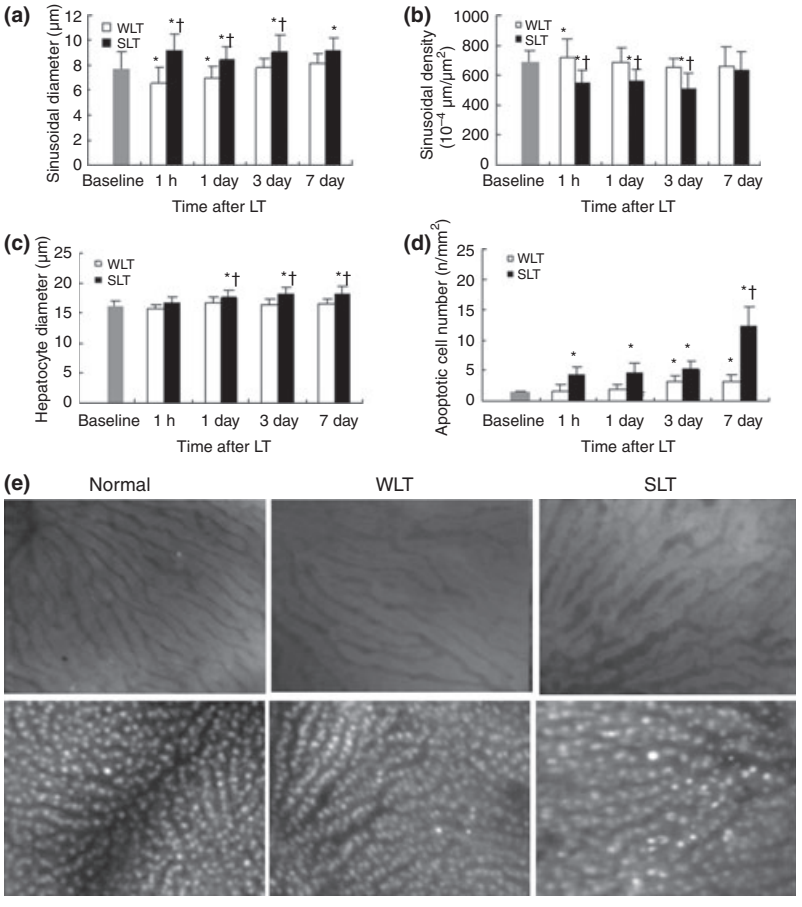


**Figure 5** Sinusoidal parameters and hepatocellular injuries after transplantation with whole and small-for-size liver grafts ($n = 5$). (a) Quantitative analysis of sinusoidal diameter. (b) Quantitative analysis of sinusoidal density. (c) Quantitative analysis of nucleo-nuclear distance along individual hepatocellular columns. (d) Quantitative analysis of hepatocytes apoptosis. WLT, whole graft liver transplantation; SLT, small-for-size liver transplantation; *$P < 0.05$, vs. baseline; †$P < 0.05$, SLT group vs. WLT group at respective time point. (e) Representative intravital fluorescence microscopic images of hepatic lobules and acinus (upper line) and hepatocytes which were stained with H33342 (lower line) in SLT group, WLT group and normal liver. Original magnification ×320.

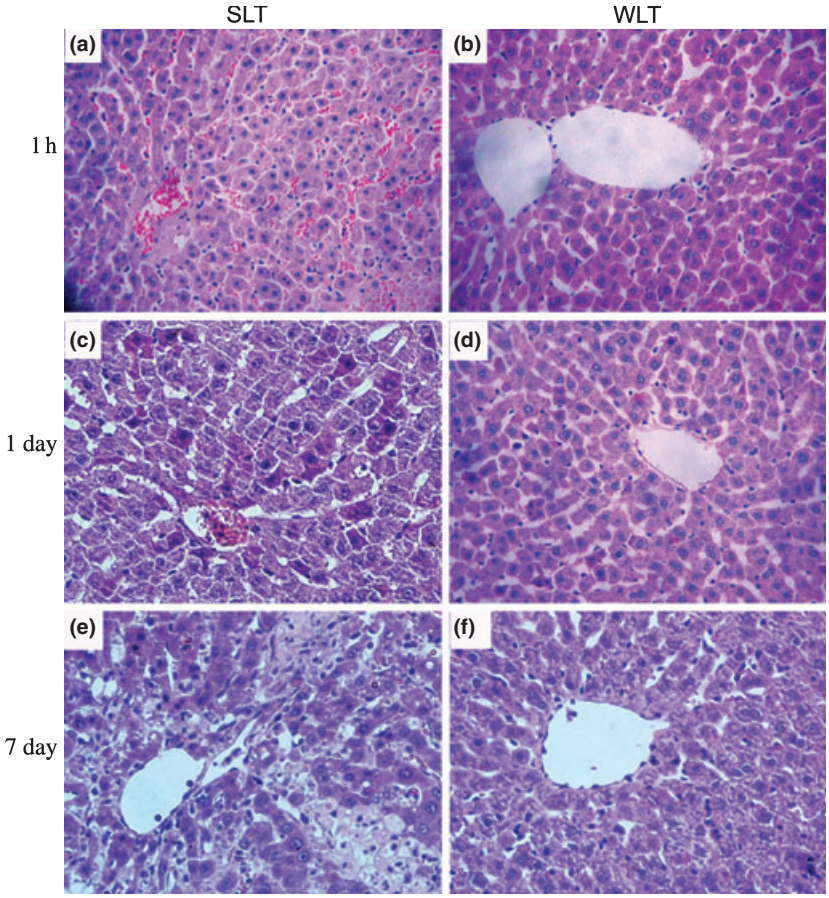


**Figure 6** Morphological alteration after transplantation with whole and small-for-size liver grafts. (a) Small-for-size graft showed apparent sinusoidal congestion at 1-h after transplantation. (c) Hepatocellular eosinophilic degeneration mainly in periportal and mid-zonal regions was showed at day 1 in SLT group. (e) More Severe disruption of lobular architecture with extensive necrosis was present at day 7 in SLT group. (b, d, and f) Normal hepatic structure was found in WLT group at day 1, 3 and 7 respectively. Original magnification ×400.

tissue, which dissected into the periportal hepatic parenchyma when severe (Fig. 6a and c). More Severe disruption of lobular architecture with extensive necrosis was present at 3d after reperfusion (Fig. 6e). In contrast, minimal damage was observed in WLT group (Fig. 6b, d and f).

Under electron microscopy, mitochondria swelling and vacuolated change in hepatocytes, sinusoidal congestion, collapse of the space of Disse and an irregular large gap between the sinusoidal lining cells was seen in the SLT group (Fig. 7a and b). Sinusoidal lining and hepatocelluar ultrastructure were intact in the WLT group (Fig. 7c and d).

## Discussion

Partial liver transplantation has been a valuable alternative in solving the problem of organ shortage [5,10,24]. However, when the graft size is below a critical limit, graft failure may develop and survival will be adversely affected. The minimum graft size for survival is about 40% of the ideal liver weight for both rats and human beings. Smaller grafts are associated with signs of liver failure [3,6,14] causing death of the recipient within a few days in the absence of retransplantation. Recent studies have showed that portal hypertension occurring during very early phase of transplantation was the key factor for graft injuries [10,13].

Apart from the common IRI, SFS grafts also suffer mechanical injury related to hemodynamic force [25]. At the time of reperfusion, excessive blood inflow (in relation to graft size) generates altered physiological state including increased portal blood flow which induces shear stress and damage to sinusoidal endothelial cells, and Kupffer's cell activation, which contributes to acute liver failure [7,14,25,26]. However, the portal hypertension was only detected during the first 60 min after reperfusion in this study and other previous articles. Man *et al.* [13] concluded that during reperfusion of small grafts the resultant transient portal hypertension causes permanent injury at the sinusoidal level that leads to progressive dysfunction or damage of the graft. However, their observation lasted only 24 h and furthermore, the majority of studies focused on the inflammatory cascades and vasoregulatory disturbance triggered by shear stress during early phase after transplantation. As the sinusoids are the

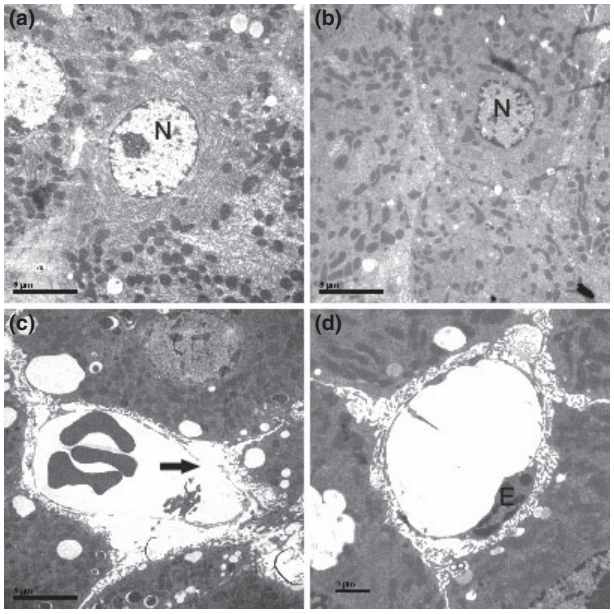


**Figure 7** Hepatic ultrastructural changes detected by electron microscopy. (a) Mitochondria swelling and vacuolar change in hepatocytes was showed in SLT group; ×6000. (b) Normal ultrastructure was showed in WLT group; ×6000. (c) Sinusoidal congestion, collapse of the space of Disse and an irregular large gap between the sinusoidal lining cells was seen in the SLT group; ×6000. (d) Sinusoidal lining was intact in the WLT group; ×8000. E = endothelial cell; N = nucleus; ↑ = sinusoidal lining cell; * = swollen mitochondria.

principal vessels involved in the transvascular exchange between blood and the parenchymal cells [27] and play an important role in hepatic microcirculation. The long-term sinusoidal microcirculatory state seems critical but has not been clearly known.

Sinusoidal perfusion rate decreased rapidly in the SLT group, indicating that the functional liver mass (FLM) was not equal to the remnant liver that has been transplanted. The importance of a reduced FLM for the onset of a SFSS was reported by Tucker and Heaton [28] and is now widely accepted clinically. Sinusoidal nonperfusion area gradually increased in the SLT group that might partially be attributable to the sinusoidal congestion and endothelial denudation when RBC squeezed into the periportal hepatic parenchyma causing sinusoidal obstruction [13,29]. Another reason might be hepatic stellate cell (HSC) constriction, which was mediated by endothelin-1 (ET-1). There is evidence suggesting that ET-1s have an effect on hepatic hemodynamics under physiological conditions [20]. Our unpublished results reported the increase of ET-1 levels in the suprahepatic vena cava from 3 h to 7 days after transplantation as well as up-regulation of ET-1 receptors, especially type A (ETAR). ET-1 promotes ETAR-mediated HSC contractility, by causing enhanced myosin phosphorylation followed by co-localization to bundles of polymerized actin, resulting in generation of contractile force [30].

Because of the elevation of nonperfusion rate, the area of hepatic vascular bed in FLM would decline relatively. Our studies also showed overperfusion state in FLM, which displayed as elevation of RBC velocity and sinusoidal dilation, although transient portal hypertension lasted only for <1 h. The imbalance of perfusion in FLM might lead to significant increases of the ratio of microvascular flow per unit weight of FLM in the SLT group. As is the case with the imbalance of ventilation/perfusion ratio in pulmonary disease, persistent abnormity of the flow/graft ratio also caused graft injuries. Therefore, severe shear stress from hyper-velocity in FLM which persisted longer than 7 days caused sustained microvascular barrier response. Furthermore, liver function would also be impaired by the shortened contact time between blood and hepatocytes resulting in dysfunction of hepatocellular metabolism, a consequence of the elevated blood flow rate [31].

The nucleo-nuclear distance of hepatocytes along the individual columns increased since 24 h after transplantation significantly, suggesting hepatocellular swelling in the SLT group. Besides, hepatocellular apoptosis was dramatically intensive under IFM. Our conventional detections such as serum biochemistry and morphological alteration also showed more grafts lesion in the SLT group especially in late phase. We speculated that deteriorated sinusoidal microcirculation played important role in graft injuries as above.

The sustained higher levels of RBC velocity and sinusoidal diameter could also occur in liver regeneration, another important issue to be considered in small-for-size liver transplantation. We have not measured graft's regenerative capability in our SLT group yet. However, portal hyperperfusion in small-for-size livers, in case of major hepatic resections or transplantation, might seriously impair postoperative liver regeneration [32] which was also reported by other researchers. Mao *et al.* [33] demonstrated that the proliferating cell nuclear antigen indices were lower after SFS liver transplantation compared with those after partial hepatectomy. Huda *et al.* [34] reported that few mitotic hepatocytes were observed in SFS graft in the absence of additional treatment. In our study, the findings of dramatic elevation of apoptotic hepatocytes in SLT were quite different from the hepatectomy situation [23] in which the number of cell apoptosis increased only slightly. The mechanism of regenerative suppression in small-for-size grafts has been demonstrated with inhibition of the c-Jun N-terminal kinase/c-Jun and CyD1 pathways [35] and with STAT3 pathways [34] all of which play a critical role in regeneration. We speculated that the increased injury and suppressed proliferation in small-size grafts further decrease the FLM and together lead to graft

dysfunction. A recent study also showed progressive hepatic sinusoidal injury even as late as 2 weeks after small graft (50%) liver transplantation [36]. Besides increasing the likelihood of liver regeneration, the remodeling of the hepatic microenvironment probably also disrupted exchange between hepatocytes and sinusoidal flow [22].

In this study, we first reported the timing and impaired pattern of sinusoidal microcirculatory hemodynamic state in small-for-size liver transplantation. Our quantitative analysis showed not only transient portal hypertension, but also long-term persistence of the microcirculatory disturbance, which could exacerbate the injury and induce graft failure. Sinusoidal microcirculatory changes reflected grafts' hemodynamic states accurately rather than transient portal hypertension. Decrease of sinusoidal reperfusion area and hyper-hemodynamics in FLM resulted in severe disturbance of hepatocytes homeostasis and remnant graft loss.

## Authorship

JL: performed research and wrote the paper. LL: designed research and wrote the paper. TM: collected data. XY: analysed data. WC: performed research. GX: performed research. TL: designed research.

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