Dynamic microcirculatory changes in liver graft from non-heart-beating donor with warm ischemia injury in rat

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BACKGROUND: **Since the 1990s, liver grafts from non-heart-beating donor (NHBD) have become an alternative because of the deficiency of grafts from heart-beating-donors (HBDs). Warm ischemia injury, however, directly influences the grafts' activity and functional recovery after operation. We investigated the microcirculatory change of liver graft at different warm ischemia time (WIT) in rats and determined the maximum limitation of liver graft to warm ischemia.**

METHODS: **According to WIT, 120 rats were divided randomly into 5 groups of 0, 15, 30, 45, 60 minutes respectively. The microcirculatory changes of their liver grafts were measured including serum level of hyaluronic acid (HA) and ultrastructural changes. After orthotopic liver transplantation (OLT), the recovery of microcirculation of the liver grafts after 24 hours, 48 hours and 3 days was observed.**

RESULTS: **Microcirculatory changes and function of the liver grafts became normal after reperfusion when the WIT was less than 30 minutes. In the 45-minute WI group, part of blood sinusoids was full of cytoplasmic blebs stemming from the microvilli of hepatocytes and hemocytes. The serum level of HA in each group after 45 minutes of WI recovered after reperfusion.**

CONCLUSIONS: **The microcirculatory change of rat liver graft is reversible when the WIT is less than 30 minutes: rat liver graft could be safely subject to warm ischemia within 30 minutes. The maximal 45 minutes of WI can be tolerated by the microcirculatory function of liver graft. After 60 minutes of WI, irreversible disturbance of microcirculation may appear.**

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KEY WORDS: **liver transplantation; warm ischemia injury; microcirculatory change**

Introduction

The shortage of donor liver is the barrier for the practice of liver transplantation. Organs from non-heart-beating donors (NHBDs) may alleviate this problem effectively. As a marginal pool of donor liver, it has drawn more and more attention in recent years. In this study, we observed dynamically endothelial function of the hepatic sinusoids and microcirculatory changes and their recovery after transplantation at different warm ischemia time (WIT).

Methods

Establishment of animal model

Healthy male adult Sprague-Dawley (SD) rats weighing 250 g to 300 g purchased from the Experimental Animal Center at Sun Yat-Sen University were used as models. Mean weight of recipient rats was a little bit heavier than that of the donor rats.

Warm ischemia

Under ether aspiration anesthesia, a midline laparotomy was performed in supine position. 0.2 ml of heparin sodium solution (1250 U heparin sodium) was injected via the dosum of the penis vein to heparinize the donor liver. Subsequently the diaphragm was sheared, the basilar part of the heart clamped, and the thoracic aorta blocked. A donor liver warm ischemia model thus was established.

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Liver transplantation

After scheduled duration of warm ischemia for each group, 20 ml of 0-4 ℃ lactic acid Ringer's solution (50 U/ml heparin sodium) was infused into the abdominal aorta of the rat via a catheter. The liver graft became fulvous when the filling solution flowed out via the sheared right atria. The liver ligaments were dissected, the pyloric vein proximal to the portal vein was ligated after the hepatic artery and portal vein were freed, the infrahepatic inferior vena cava was isolated, and the right suprarenal and right renal veins were severed. The suprahepatic inferior vena cava close to the diaphragmatic anulus, the hepatic artery, the portal vein in the confluence of the portal and splenic veins, the infrahepatic inferior vena cava over the left renal vein were cut off. Specimens of donor livers were preserved in the 4 ℃ lactic acid Ringer's solution. Anesthesia, operative position and incision were the same in donors and recipients. The angioanastomotic techniques were modified from bicuff techniques suggested by Kamada and Sun.[1,2] Cold ischemia (CI) including cold douche, donor liver fitting, and cold preservation lasted 50±3.5 minutes, and the anhepatic period was 20±2.5 minutes.[3]

Groups and materials

One hundred and twenty SD rats were randomly divided into 5 groups of WI for 0, 15, 30, 45, 60 minutes respectively. OLT was performed in each group (12 donors and 12 recipients). The level of serum hyaluronic acid (HA) was dynamically observed at 24 hours, 48 hours, 3 days and 5 days after transplantation. The ultrastructure of the hepatic sinusoids was observed at 24 hours, 48 hours and 3 days after transplantation.

Indexes and methods

Serum HA

The level of HA was measured radioimmunologically, with the test kit provided by the Shanghai Haiyan Bio-medical Technique Center, Shanghai, China.

Ultrastructure of the hepatic sinusoids

At different time of WI, the observed hepatic tissues were cut into pieces of 1 mm^3, of which 5 were taken randomly as specimens. Sections of these specimens were observed under a transmission electron microscope and a scanning electron microscope.

Statistical analysis

The data were expressed as mean ± standard deviation. With a SPSS 10.0 package, the results were tested by analysis of variance (ANOVA) including Student-Neuman-Keuls procedure. A P value less than 0.05 was regarded as significantly different.

Results

Dynamic change of serum HA

Twenty-four hours after transplantation, the level of HA increased sharply with the prolongation of WI. Compared with the 0-minute WI group, the level of HA in groups of WI less than 45 minutes showed a recovery ($P>0.05$), but it was hard to recover in the 60-minute WI group ($P<0.05$, Table).

Ultrastructure of the hepatic sinusoids

The 0-minute WI group

Normal sinusoids were large and irregular without accumulation of hemocytes. Endothelial cells were similar to endothelial cells of the capillaries. Under the scanning electron microscope, gaps of 0.1-0.5 μm were seen between the endothelial cells. The cells themselves appeared to be highly fenestrated, with a specific structure or "endothelial sieve plate" (Figs. 1 and 2). The basal lamina around the sinusoids was incomplete, without morphological barrier between the sinusoids and the perisinusoidal space. Then, the microvilli and Disse space were observed through the endothelial gaps. After reperfusion, some endothelial cells became slightly edematous but recovered rapidly.

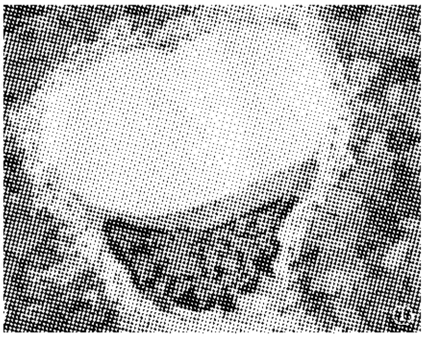
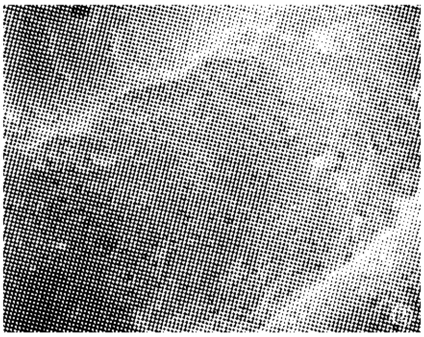
The 15- and 30-minute WI groups

The endothelial gaps enlarged in the warm ischemia period. Twenty-four hours after reperfusion, cytoplasmic blebs stemming from the microvilli of hepatocytes bulged into the sinusoids, and 48 hours after reperfusion, the sinusoids returned to normal without accumulation of hemocytes.

Table. HA dynamic changes after reperfusion (ng/ml)

Group	24 h after reperfusion	48 h after reperfusion	3 d after reperfusion	5 d after reperfusion
0-minute WI	1246.5±66.5	935.7±33.9	237.3±28.6	223.7±16.9
15-minute WI	1967.5±186.6	1088.3±78.7	259.6±33.7 *	231.6±23.8
30-minute WI	3085.2±329.8	1937.5±230.2	269.2±31.9 *	254.6±26.9
45-minute WI	4025.8±384.2	2672.2±344.3	279.8±36.6 *	236.9±31.6
60-minute WI	5244.6±573.5	5373.2±484.2	4339.6±639.8△	4962.6±520.6

Compared with the 0-minute WI group. *: HA recovered 3 days after reperfusion in groups with WI less than 45 minutes ($q'=2.511, P>0.05$); △: HA could hardly recovered 3 days after reperfusion in the 60-minute WI group ($q'=27.162, P<0.001$).

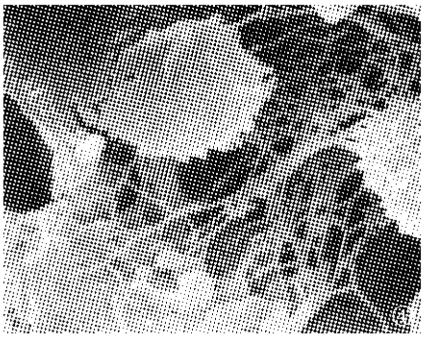
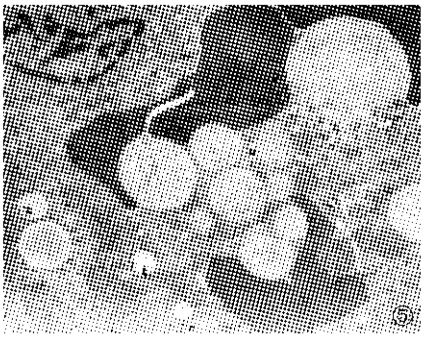
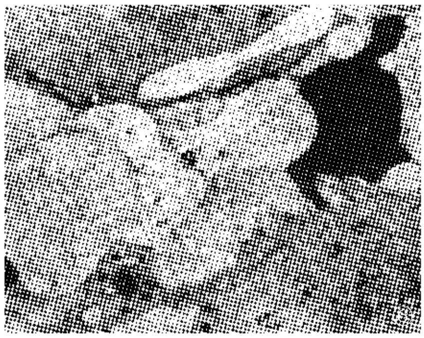




Fig. 1. The 0-minute WI group. Normal ultrastructure of sinusoids in liver graft (transmission electron microscope ×3000).

Fig. 2. The 0-minute WI group. Endothelial sieve plate of sinusoids in liver graft (scanning electron microscope ×6000).

Fig. 3. The 45-minute WI group. After reperfusion, some sinusoids were full of cytoplasmic blebs and irregular endothelial sieve plate (scanning electron microscope ×6000).

Fig. 4. The 60-minute WI group. After reperfusion, sinusoids were full of cytoplasmic blebs, reticular fibrosis, and hemocytes (scanning electron microscope ×6000).

Fig. 5. The 45-minute WI group. After reperfusion, swollen endothelial cells and bleb-like swollen subcellular structure were noted (transmission electron microscope ×5000).

Fig. 6. The 60-minute WI group. After reperfusion, bleb or balloon-like swollen endothelial cells were seen with blocked sinusoids. Thus the microcirculation underwent irreversible disturbance (transmission electron microscope ×5000).

The 45-minute WI group

In the warm ischemia period, edema of endothelial cells was noted with larger and mingled endothelial gaps. Twenty-four hours after transplantation, more and larger cytoplasmic blebs were seen. Forty-eight hours after reperfusion, most sinusoids became normal, but some showed accumulation of cytoplasmic blebs and irregular endothelial sieve plate (Fig. 3).

The 60-minute WI group

In the warm ischemia period, accumulated cytopalsmic blebs and hemocytes in the sinusoid were observed with large and irregular endothelial gaps and vacuoles in the cytoplasm of edematous cells. Twenty-four and 48 hours after reperfusion, the membrane of the cells was ruptured with karyopyknosis and karyorrhexis. Most of endothelial cells underwent necrosis and shedding, and lots of hepatic sinusoids were full of cytoplasmic blebs, reticular fibrosis, and hemocytes (Fig. 4). Scanning electron microscopy showed bleb or balloon-like swollen endothelial cells and abstracted sinusoids which indicate the irreversible disturbance of microcirculation (Figs. 5 and 6).

Discussion

Liver transplantation has been an effective method in the treatment of end-stage hepatic diseases, but the shortage of donor liver is a pressing problem. To alleviate the disparity between donor organs and recipients, surgeons reconsider the use of liver from NHBD. How to evaluate the quality of liver graft and how to ascertain its time limit of safety for warm ischemia[4] are of paramount importance in liver transplantation. In the present study hepatic injury was reversible within 30 minutes of warm ischemia and the function of liver graft became normal.[5-7]

Microcirculatory disturbance after warm ischemia injury is not adequently reported;[8-12] but it is thought to be an important mechanism of injury caused by warm ischemia reperfusion in liver transplantation. Hepatocytes may be damaged in varied degree after warm ischemia reperfusion. The level of HA metabolized mainly in hepatic endothelial cells may reflect endothelial function. Hepatic sinusoids comprise microcirculatory capillaries with specific inner walls because of the absence of basal lamina and septal support in the endothelium.

In this study, sinusoid endothelial cells were sensitive to warm ischemia, and the endothelium was injured at both the warm ischemia and reperfusion stages. Twenty-four hours after reperfusion, the levels of serum HA in each group increased markedly. Compared with the 0-minute WI group, the level of HA in groups with WI less than 45 minutes recovered to normal 3 days after reperfusion ($P>0.05$), but in the 60-minute WI group, it could hardly recover ($P<0.05$). Ultrastructurally, some changes at early stage such as enlarged endothelial gaps and edematous microvilli were noted in groups with WI less than 30 minutes. In the 45-minute WI group, swollen endothelial cells, enlarged and mingled endothelial gaps were observed. Irreversible changes such as chromatin margination, karyopyknosis, membrane rupture and karyorrhexis appeared in the 60-minute WI group. The degree of warm ischemia-reperfusion injury was dependent on the duration of warm ischemia. Damage to microcirculation was severer 24 hours after reperfusion. Forty-eight hours after reperfusion, sinusoids recovered without accumulation of hemocytes in groups with WI less than 30 minutes. In the 45-minute WI group, most sinusoids recovered except some showed accumulation of cytoplasmic blebs, and irregular endothelial sieve plate. In the 60-minute WI group, however, damage to the endothelium could hardly be improved with blocked sinusoids, bleb or balloon-like endothelial cells, thus resulting in irreversible disturbance of microcirculation.

The present study indicated that prolonged warm ischemia may cause persistent injury of the hepatic sinusoids and endothelium, even damage to microcirculation, which induces sinusoids blockage and reperfusion disturbance. After warm ischemia injury, endothelial gaps are enlarged and mingled, and cytoplasm stemmed from edematous microvilli may extrude into the sinusoid and block the microcirculation. At the same time, the endothelial gaps became enlarged, and endothelial sieve plate ruptured. The endothelium without septal support and basal lamina is apt to break off and block the sinusoid during reperfusion. Adhesion of neutrophiles, platelets, and hypoxia injury to endothelial cells would cause microthrombosis, another mechanism for sinusoid blockage. The endothelium may be damaged by some lymphokines and oxidative free radicals induced by lymphocytes, leukocytes, and Kupffer cells in the sinusoids. Moreover, metabolic disturbance could insult the normal cellular and subcellular structure and function, and cells undergo edema, degeneration, necrosis, and rupture, which also aggravate sinusoid blockage.

We conclude that microcirculatory change of liver graft can be reversed after reperfusion when WI is less than 30 minutes. Rat liver graft is reversible when WI is less than 30 minutes. The duration of 45 minutes of WI could be maximal for the tolerance of the function of microcirculation of liver graft. After 60 minutes of WI, the microcirculatory disturbance is irreversible.

Competing interest

The author or authors do not choose to response to the statements listed in Instructions for Authors.

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