

Influence of warm ischemia injury on hepatic functional status and survival of liver graft in rats

Xiao-Shun He, Yi Ma, Lin-Wei Wu, Wei-Qiang Ju, Gui-Hua Chen,
Rui-De Hu and Jie-Fu Huang

Guangzhou, China

OBJECTIVES: To investigate the changing patterns of functional and histological status, observe the posttransplantation survival of liver graft under different warm ischemia time (WIT) in rats, and determine the maximum limitation of liver graft to warm ischemia.

METHODS: According to WIT, the rats were randomized into 7 groups, with WIT of 0, 10, 15, 20, 30, 45, 60 minutes respectively. Serum concentrations of alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase were measured at 1, 2, 3 and 5 days after orthotopic liver transplantation respectively. Liver graft specimens were observed histopathologically at the same interval. The rats' survival in each subgroup was observed.

RESULTS: In terms of graft survival, there was no significant difference between subgroups within 30-minute WIT. In the group with 30-minute WIT, the recipient rats' survival rate was 83.3% (10/12) at one week, 58.3% (7/12) at one month, and 50.0% (6/12) at 3 months. In the group with 45-minute WIT, the recipient rats' survival rate was 66.7% (8/12) at one week, 33.3% (4/12) at one month, and 8.3% (1/12) at 3 months, whereas only 8.3% (1/12) of the rats had one-week survival in the group with 60-minute WIT.

CONCLUSIONS: These results indicate that rat liver graft could be safely subject to warm ischemia within 30 minutes. When WIT is prolonged to 45 minutes, the recipients long-term survival is severely insulted, and both function and histological structure of liver graft may develop irreversible damage when WIT is prolonged to 60 minutes.

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Key words: liver transplantation; warm ischemia injury; survival

Introduction

From the 1990s, liver transplantation has become an effective management in the treatment of end-stage liver diseases, but the shortage of do-

nor liver is a critical limitation for liver transplantation. Organs from non-heart-beating donors (NHBD) seem to be an option to alleviate this problem effectively. However, the main obstacle to the use of liver from NHBD is warm ischemia to the liver related to cardiac arrest. Moreover, in liver transplantation, the allograft sustains inevitable cold ischemia in addition to rewarming injury during liver reperfusion. The quality of liver graft is a key factor for liver transplantation. It is a main unfathomed problem how to evaluate the quality of liver grafts and how to ascertain the safety time limit for warm ischemia of liver grafts. In the present study

From the Organ Transplantation Center, First Affiliated Hospital of Sun Yat-Sen University, Guangzhou 510080, China (He XS, Ma Y, Wu LW, Ju WQ, Chen GH, Hu RD and Huang JF)

Correspondence: Xiao-Shun He, MD, Organ Transplantation Center, First Affiliated Hospital of Sun Yat-Sen University, Guangzhou 510080, China (Tel: 86-20-87306082; Fax: 86-20-87306082; Email: xshe@gdmet.com)

we established a warm ischemia model and investigated the liver function and histomorphological changes 24 hours, 48 hours, 3 days and 5 days after transplantation with special emphasis on the long-term survival.

Methods

Animals and groups

Three hundred and thirty-six healthy male adult Sprague-Dawley rats weighing 250 g to 300 g from Experimental Animal Center at Sun Yat-Sen University were used. The mean weight of recipient rats was a little heavier than that of donor rats. 336 SD rats were randomly divided into two groups: 168 for donors and 168 for recipients. The donors were then randomly divided into two subgroups: 84 for prolonged observation, including spirit, activity, and survival time and the other 84 rats for investigation of liver function and histomorphological dynamic changes. Both groups were divided into 7 subgroups according to WIT 0 minute, 10 minutes, 15 minutes, 20 minutes, 30 minutes, 45 minutes, and 60 minutes, 12 animals for each group. Liver transplantation and postoperative follow-up were performed respectively.

Establishment of animal model

Warm ischemia was induced by clamping the basilar part of the heart and the thoracic aorta of the donor animals was blocked after they received 0.2 ml heparin sodium solution (1250 U heparin sodium) via the dorsum of the penis vein to establish a non-heart-beating donor model. Then the liver graft was isolated, perfused in situ through the abdominal aorta with 20 ml chilled lactic acid Ringer's solution, and subsequently stored in a bath of cold lactic acid Ringer's solution before transplantation. Immediately before clamping of the portal vein, orthotopic liver transplantation was performed according to the techniques described by Kanda and Sun^[1,2] with some modification.^[3] Cold ischemia time (CIT) was 50 ± 3.5 min, and anhepatic period was 20 ± 2.5 min.

Statistical analysis

Table. Animal survival rate under different WIT (%)

Group (min)	1-week survival rate	1-month survival rate	3-month survival rate
0	91.7 (11/12)	83.3 (10/12)	83.3 (10/12)
10	83.3 (10/12)	66.7 (8/12)	66.7 (8/12)
15	83.3 (10/12)	58.3 (7/12)	50.0 (6/12)
20	75.0 (9/12)	58.3 (7/12)	58.3 (7/12)
30	83.3 (10/12)	58.3 (7/12)	50.0 (6/12)
45	66.7 (8/12)	33.3 (4/12)* Δ	8.3 (1/12)* Δ
60	8.3 (1/12)* Δ	0.0 (0/12)*	0.0 (0/12)*

*: Compared with 0-minute WIT group, $P < 0.05$ (Fisher's exact test); Δ : compared with the previous groups, $P < 0.05$ (Fisher's exact test).

Measurement data (mean \pm SD) were tested by analysis of variance (ANOVA including Student-Neuman-Keul procedure). Enumeration data were analyzed by the chi-square test and Fisher's exact test. One-week, 1-month and 3-month survival rates between each group were compared. The data were disposed with SPSS package and a P value less than 0.05 was considered significant.

Results

Animal survival

One-week, 1-month and 3-month postoperation survivals for each group are shown in Table. There was no significant difference between the groups with WIT less than 30 minutes ($P > 0.05$). One-week survivals in groups of 0-minute and 60-minutes WIT were significantly different ($P < 0.05$). The difference was also seen in 1-month and 3-month survivals between groups 45-, 60-, 0-minute WIT ($P < 0.05$).

Liver function

Twenty-four hours after transplantation, the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) increased markedly with the elongation of WIT, and the following recovery change showed a step-like pattern. The levels of AST and ALT in groups with WIT less than 30 minutes decreased to the normal level 3 days after transplantation, and the recovery course took 5 days in the 45-minute WIT group (Figs. 1 and 2). The level of LDH in groups with WIT less than 45 minutes recovered to

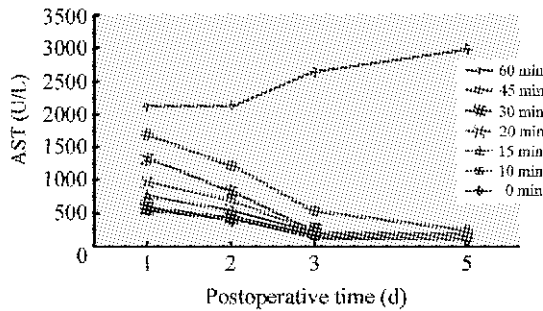


Fig. 1. Recovery change of AST after transplantation in different WIT groups. AST recovered to normal 3 days after transplantation in groups with WIT less than 30 minutes ($P > 0.05$); AST recovered to normal 5 days after transplantation in groups with WIT less than 45 minutes ($P > 0.05$); AST could hardly recover in the 60-minute WIT group ($P < 0.05$).

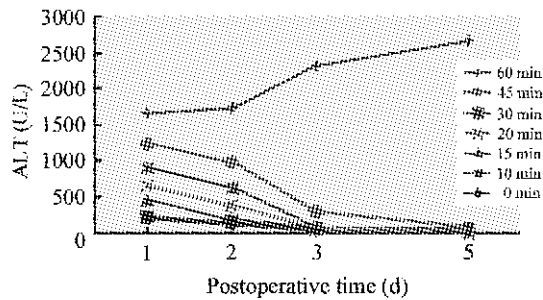


Fig. 2. Recovery change of ALT after transplantation in different WIT groups. ALT recovered to normal 3 days after transplantation in groups with WIT less than 30 minutes ($P > 0.05$); ALT recovered to normal 5 days after transplantation in groups with WIT less than 45 minutes ($P > 0.05$); ALT could hardly recover in the 60-minute WIT group ($P < 0.05$).

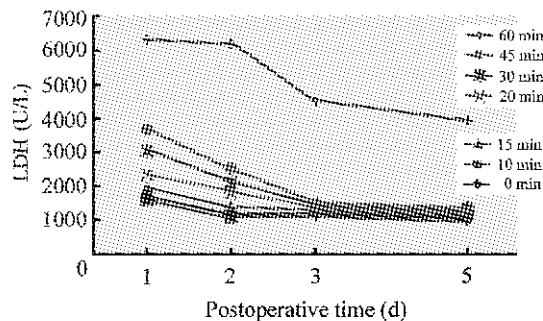


Fig. 3. Recovery change of LDH after transplantation in different WIT groups. LDH recovered to normal 5 days after transplantation in groups with WIT less than 45 minutes ($P > 0.05$); LDH could hardly recover in the 60-minute WIT group ($P < 0.05$).

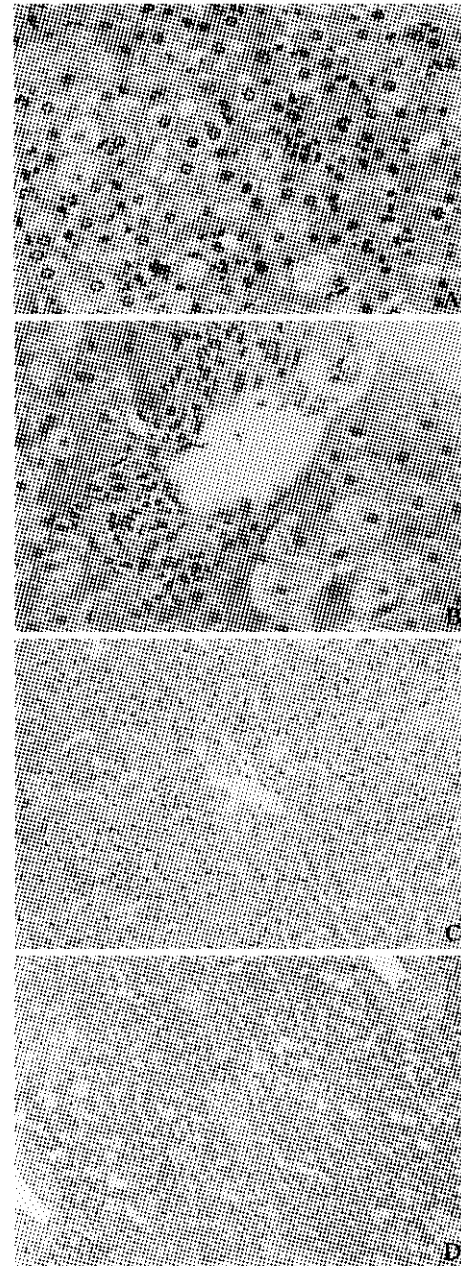


Fig. 4. Histomorphologic changes after transplantation. A: groups with WIT less than 30 minutes, cytoplasm loosening, cell edema, focal vacuole degeneration after reperfusion (original magnification $\times 200$); B: 30-minute WIT, obvious cellular edema, ballooning-like degeneration after reperfusion (original magnification $\times 400$); C: 45-minute WIT, focal necrosis around the lobule central area after reperfusion (original magnification $\times 100$); D: 60-minute WIT, plaque-like area necrosis after reperfusion (original magnification $\times 40$).

the normal level 3 days after transplantation, but enzymes in the 60-minute WIT group could hardly recover to normal (Fig. 3).

Histomorphological and subcellular structure

Histomorphological and subcellular structural changes constituted a dynamic process. Microscopically, both histomorphological and subcellular structures remained integrated after OLT when WIT was 0 minute. Vacuole degeneration and cellular edema were seen after reperfusion in groups with WIT less than 30 minutes, especially in the lobule center area. In the 30-minute WIT group, ballooning degeneration and infiltration were seen with obvious acidophilus. These changes were reversible and significant 48 hours after reperfusion. When WIT prolonged to 45 minutes, cell degeneration aggravated with focal necrosis, which was first seen in the lobule center area. In the 60-minute WIT group, large area or diffused irreversible necrosis presented (Fig. 4).

Discussion

The shortage of donor liver is a critical limitation for liver transplantation in treatment of end-stage liver diseases. Organs from non-heart-beating donors (NHBD) seem to be optional to alleviate this problem effectively, and this marginal pool of donor livers has drawn more and more attentions. Warm ischemia injury in liver transplantation is a complicated pathophysiologic process, in which cells will undergo a series of metabolism, structural and functional injury. In the present study, we explored the safe limitation for warm ischemia of liver grafts and the relationship between WIT and post-operative survival, aiming to find an effective way to alleviate the problem of donor shortage and save more patients with end-stage liver diseases.

To ascertain the time limitation for warm ischemia in liver lobe resection, some research found that livers of humans and pigs can tolerate 60 minutes of warm ischemia injury.^[3] However, warm ischemia injury to liver grafts from NHBD is unlike the process in liver resection, because the grafts would inevitably sustain further injury in the

period of preservation, operation and reperfusion. The presentation of UW solution and the improvement of operative skills have led to a reconsideration of the use of marginal pools such as NHBD.

NHBD programs have been used successfully in kidney transplantation, and are able to increase donor pool of kidneys by 40%. In the field of liver transplantation, the use of livers from NHBD shows less favored clinical results because of the limited hepatic tolerance to warm ischemic damage compared with kidneys. Several clinical and experimental studies have shown that liver can compensate 60-minute WIT during liver resection,^[4] but few studies focused on the limited period of warm ischemia before transplantation. And these studies didn't take the long-time survival as an index to evaluate the quality of liver grafts,^[5,6] which is very important in clinical practice.

In the present study, the levels of AST, ALT and LDH increased markedly with the elongation of WIT 24 hours after transplantation. The following recovery change showed a step-like pattern. The levels of AST and ALT in groups with WIT less than 30 minutes decreased approximately to the normal level 3 days after transplantation and the recovery course took 5 days in the 45-minute WIT group. The level of LDH in groups with WIT less than 45 minutes recovered to normal 3 days after transplantation, but that of enzymes in the 60-minute WIT group could hardly recover to normal. Analysis of long-term survivals showed that rat livers can tolerate WIT for 30 minutes in groups with WIT less than 30 minutes. One-week, 1-month and 3-month survival rates were 83.3% (10/12), 58.3% (7/12) and 50% (6/10), respectively. No significant difference was seen compared with the 0-minute WIT group (HBD group). In the 45-minute WIT group, the 1-week survival rate was 66.7% (8/12), which was not different from that of the HBD group, but the 1-month and 3-month survival rates were 33.3% (4/12) and 8.3% (1/12). The difference was significant compared with the HBD group, showing that the long-time survival had been insulted. In the 60-minute WIT group, the 1-week survival rate was only 8.3% (1/12).

Histological study showed that histological and

subcellular structure underwent a dynamic process in a time-dependent manner. When WIT was limited to 30 minutes, vacuole degeneration and cellular edema were noted after reperfusion. When WIT prolonged to 45 minutes, cell degeneration aggravated with focal area, especially in the lobule center area. These changes were consistent with those found by liver graft function tests.

In terms of graft survival, the results showed that survival time in groups with WIT less than 30 minutes had no significant difference. In the 45-minute WIT group, the animals might survive the early postoperative period, but the long-term survival was severely insulted when WIT prolonged to 60 minutes. Both functional and histological structure of liver graft would develop irreversible damage and no recipients could survive.

Competing interest

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

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