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• BASIC RESEARCH •

Safe time to warm ischemia and posttransplant survival of liver graft from non-heart-beating donors

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Abstract

AIM: To explore the dynamical changes of histology, histochemistry, energy metabolism, liver microcirculation, liver function and posttransplant survival of liver graft in rats under different warm ischemia times (WIT) and predict 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 min, respectively. The recovery changes of above-mentioned indices were observed or measured after liver transplantation. The graft survival and postoperative complications in each subgroup were analyzed.

RESULTS: Liver graft injury was reversible and gradually resumed normal structure and function after reperfusion when WIT was less than 30 min. In terms of graft survival, there was no significant difference between subgroups within 30 min WIT. When WIT was prolonged to 45 min, the recipients' long-term survival was severely insulted, and both function and histological structure of liver graft developed irreversible damage when WIT was prolonged to 60 min.

CONCLUSION: The present study indicates that rat liver graft can be safely subjected to warm ischemia within 30 min. The levels of ATP, energy charge, activities of glycogen, enzyme-histochemistry of liver graft and its recovery potency after reperfusion may serve as the important criteria to evaluate the quality of liver graft.

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INTRODUCTION

Attributed to the widespread applications of effective immunosuppressants and the prefect perioperative management, liver transplantation has achieved a great success during the past 40 years, but the shortage of liver donors has limited its clinical usage greatly. Quality of liver graft is a key factor for liver transplantation. Organs from NHBD (non-heart-beating donors) seem to be an option to alleviate the problem of liver donor shortage effectively[1]. However, warm ischemia to the liver related to cardiac arrest remained a main obstacle to the use of livers from NHBD[2-4]. Moreover, in liver transplantation, the allograft sustains inevitable cold ischemia in addition to rewarming injury during liver reperfusion[5]. Therefore, warm ischemia-reperfusion injury of liver grafts has become a hot topic with theoretical and clinical significance, and it has drawn more and more attention[1]. It is a main unfathomed problem of how to evaluate the quality of liver grafts and how to ascertain the safety time limit for warm ischemia of liver grafts. The outcomes of this research were rather different mainly because the previous researches focused on the relationship between warm ischemia time (WIT) and the short-time survival, neglecting the long-term survival and complications. We investigated the dynamical changes of histology, histochemistry, energy metabolism, microcirculation, especially focusing on liver function and posttransplant survival of liver graft, under different WIT to establish the predictive limitation of liver graft to warm ischemia injury, which might be helpful for the further research in similar clinical situation.

MATERIALS AND METHODS

Establishment of animal model

Adult healthy male Sprague-Dawley rats, weighing 250-300 g, supplied from Experimental Animal Center in Sun Yat-Sen University, were used for the models. Breeding conditions were in coincidence with SPF (specific pathogen free animal) standards. Mean weight of recipient rats was a little heavier than that of donor rats.

Animal model of warm ischemia A midline laparotomy was performed in supine position after an ether aspiration anesthesia, 0.2 mL of heparin sodium solution (1 250 U heparin sodium) was injected via dorsum of penis vein to heparinize the donor liver. Thereafter, we sheared diaphragm, clamped the basilar part of the heart and blocked the thoracic aorta. Thus, a donor liver warm ischemia model was established.

Animal model of liver transplantation After the predicted warm ischemia duration in each group, 20 mL of 0-4 ℃ lactic acid ringer's solution (50 U/mL heparin sodium) was infused into the abdominal aorta via a catheter. The liver graft turned fulvous when filling solution flowed out via the sheared right atria. All of the liver ligaments were dissected, the pyloric vein was ligated proximal to the portal vein after the hepatic proper artery and portal vein were freed, the infra-hepatic inferior vena cava was isolated and the right suprarenal vein and right renal vein were cut. The supra-hepatic inferior vena cava was cut in the position close to the diaphragm anulus, the hepatic artery was ligated and cut, the portal vein was cut in the confluence of portal vein and splenic vein, the infra-hepatic inferior vena cava was cut over the left renal vein. Specimens of donor liver were preserved in the 4 ℃ lactic acid ringer's solution. Anesthesia, position and incision were all the same in donors and recipients' operations. We modified the angio-anastomotic technique[6] on the basis of cuff technique suggested by Kamada *et al*.[7] and Sun *et al*.[8].

Cold ischemia time (CIT) was 50±3.5 min, anhepatic phase was 20±2.5 min.

Group and observation index

Three hundred and seventy eight SD rats were used in our study. Forty two (6 in each group with WIT of 0, 10, 15, 20, 30, 45 and 60 min) were used for the observation of histology, histochemistry, ultrastructure and metabolism change after only warm ischemia injury; other 336 rats were performed with orthotopic liver transplantation according to the "modified cuff method"[6]. Among the 336 rats, 168 were used for donors and 168 for receivers. The donor group was randomly divided into 2 subgroups: 84 for prolonged survival observation, including spirit, activity, complications, death diagnosis and mean survival; the other 84 rats for investigation of the dynamical changes of liver function, histology, histochemistry, microcirculation, energy metabolism and ultrastructure (transmission electron microscope and scanning electron microscope). Both groups were divided into 7 subgroups with WIT of 0, 10, 15, 20, 30, 45 and 60 min, each subgroup consisted of 12 rats. Liver transplantation and postoperative follow-up were performed respectively. Careful attention was paid to the fluid replacement after blood withdrawal.

Statistics analysis

Data were expressed as mean±SD. Analysis of variance (ANOVA including SNK-*q* test) was used to analyze the data. Enumeration data were analyzed by Chi-square test and Fisher test. The activity and distribution of succinic dehydrogenase (SDH), cytochrome oxidase (CO) and ATPase, were respectively observed under microscope in a semi-quantitative way. Kaplan-Meier analysis was applied for the relationship between WIT and survival, while correspondence analysis was applied for that between WIT and complication. All the statistical procedures were performed with SPSS package and $P<0.05$ was considered statistically significant.

RESULTS

Survival situation

In groups with WIT of 0, 10, 15, 20, 30, 45 and 60 min, the median survival time was 140.5, 132.5, 76, 109, 58, 13.5 and 3 d, respectively (Figure 1). One week, 1-mo and 3-mo postoperation survivals of each group are shown in Table 1. There was no significant difference between groups with WIT less than 30 min. One week survival in WIT of 0 min and 60 min group was significantly different ($P<0.05$). The difference was also significant between 45, 60 and 0 min in 1-mo and 3-mo survival ($P<0.05$)[9].

Dynamic histological and subcellular structure observation

The histological and subcellular structure change was a dynamic process[10]. Histological structure changed slightly when WIT was less than 30 min. Cytoplasm loosening, cells edema and focal vacuole degeneration were noted when WIT was over 30 min, especially in the lobule center area, leukocytes infiltration was noted in the portal area, acidophilus was obvious in some hepatocytes. The above pathologic changes aggravated when WIT elongated to 60 min, cell degeneration was diffuse or extended to a focal area, and even lipid degeneration could be seen. The degree of degeneration was depending on the duration of WIT, but necrosis could hardly be observed under light microscope. Six hours and 24 h after reperfusion, injury to liver graft became severer; in WIT 30 min group, hepatic cells presented obvious edema and some ballooning denegation; in WIT 45 min group, focal like necrosis could be noted, which was presented first in the lobule center area, the change aggravated with the elongation of WIT. Forty eight hours after reperfusion, hepatic injury resumed gradually in groups with WIT less than 45 min, while in WIT 60 min group, hepatic cells presented plaque or diffuse necrosis and the pathologic change was irreversible[11].

Electroscopically, swollen mitochondrion was noted and glycogen increased 24 h post liver transplantation in groups with WIT less than 30 min. When WIT was over 45 and 60 min, more swollen mitochondrion, vacuole degeneration, broken rough endoreticular, drop of nucleoprotein and hepatic apoptosis were observed, and cells necrosis could be noted with karyopyknosis, karyorrhexis and karyolysis. Thus, the subcellular structure then underwent irreversible injury. In addition, in WIT 45 min and 60 min groups, hepatic cells and endothelial cells presented apoptosis increasingly, and cells apoptosis and necrosis could be noted simultaneously[12,13].

Table 1 One week, 1-mo and 3-mo survival rate under different WIT (*n*,%)

Warm ischemia time (min)	1-wk survival rate	1-mo survival rate	3-mo 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)[a,c]	8.3 (1/12)[a,c]
60	8.3 (1/12)[a,c]	0.0 (0/12)[a]	0.0 (0/12)[a]

[a]$P<0.05$ *vs* WIT 0 min group (*Fisher's* exact test); [c]$P<0.05$ *vs* groups WIT less than 45 min (*Fisher's* exact test).

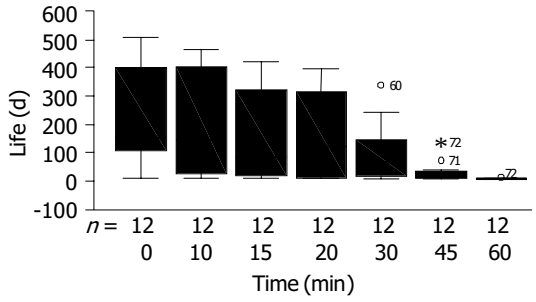

Figure 1 Statistical analysis of survival time.

Dynamic changes of histochemistry

The activities of SDH, CO, ATPase and content of glycogen decreased gradually after different WIT in a time-dependent manner, and especially significant over 30 min. The activities of SDH, CO, ATPase and content of glycogen of liver graft significantly decreased in 45 min and 60 min groups[14].

Hepatic glycogen and enzyme activities were positively related to warm ischemia time in a time-dependent manner during the reperfusion period. In WIT 15 and 30 min groups, periodic acid-Schiff reaction (PAS reaction) and enzyme histochemical activities showed a recovery potency. While in WIT 45 and 60 min groups, the liver graft underwent an irreversible injury; therefore, no evident recovery potency was found 24 h after implantation.

Microcirculation change patterns of liver graft

The microcirculation changes of liver graft were measured by serum hyaluronic acid (HA) and ultrastructural observation. The microcirculation of liver graft injury could be gradually resumed normal after reperfusion when WIT was less than 30 min. In the WIT 45 min group, part of blood sinusoids were full of the cytoplasmic blebs stemming from the microvilli of

hepatocytes and hemocytes accumulated there; the level of serum HA of each group within 45 min of WIT would almost recover after reperfusion[15].

Measurement of energy metabolism

ATP, TNA (Total adenine nucleotides) and EC in all groups were decreased dramatically after warm ischemia injury[16,17]. ATP in WIT 10, 15, 20, 30, 60 min groups was decreased to 58.7%, 34.7%, 30.1%, 20.5%, 15.3%, and 9.3% of that in WIT 0 min group, respectively. Twenty-four hours after transplantation, ATP, TNA and EC showed a tendency of recovery potency. ATP, TNA and EC had no significant difference between groups with WIT less than 30 min and WIT 0 min group. But when WIT was over 45 min the observation showed significant difference, which also could be seen between the WIT 45 min and 60 min groups. Forty-eight hours after transplantation, the above mentioned indexes recovered close to the normal level, there was no difference between groups with WIT less than 45 min and WIT 0 min group, while the indexes in group with WIT 60 min were still different from those in WIT 0 min group and WIT 45 min group[18].

Liver function

Twenty-four hours after transplantation, AST, ALT and LDH increased sharply with the elongation of WIT, the following recovering change showed a step-like pattern[19,20]. AST and ALT in groups with WIT less than 30 min decreased close to the normal level 3 d after transplantation, and the recovering course took 5 d in WIT 45 min group. LDH in groups with WIT less than 45 min recovered to the normal level 3 d after transplantation, while enzymes in WIT 60 min group could hardly recovered to the normal[21].

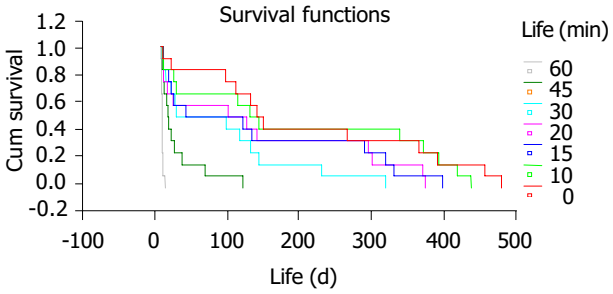

Figure 2 Survival curve (Kaplan-Meier method).

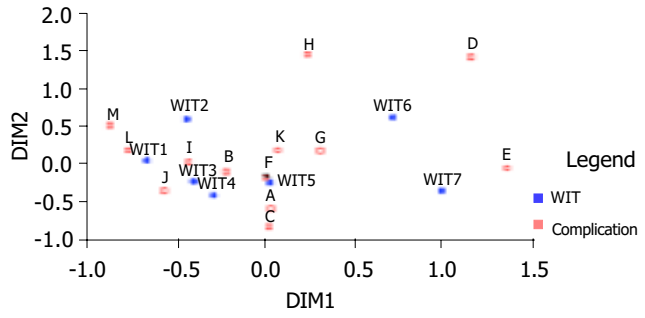

Figure 3 Relationship between WIT and complications (correspondence analysis). WIT 1, 2, 3, 4, 5, 6 and 7 represent WIT 0 min, 10 min, 15 min, 20 min, 30 min, 45 min and 60 min, respectively. A: Hemorrhage; B: SVC (superior vena cava) thrombosis; C: IVC (inferior vena cava) thrombosis; D: PV (portal vein) thrombosis; E: Liver dysfunction; F: Pulmonary infection; G: Abdominal infection; H: Bile leakage; I: Bile obstruction; J: Adhesive intestinal obstruction; K: Liver abscess; L: Unexplained; M: Natural death.

Relationship between WIT and survival

The survival curves are demonstrated in Figure 2. Survival time in groups with WIT less than 30 min had no statistical difference. Though short-term survival might be possible in WIT 45 min group, warm ischemia injury had insulted the long-term survival of liver graft. And in WIT 60 min group, the liver graft underwent irreversible injury.

Relationship between WIT and complication

The relationship between WIT and complications was illustrated by correspondence analysis (Figure 3). Complications in groups with WIT less than 30 min were similar, the incidence of liver graft dysfunction increased with the elongation of WIT and the incidence of biliary blockage was positively related to the survival time of recipient animals[22-24].

DISCUSSION

In the past 40 years, liver transplantation has achieved a great success and become the most effective method to treat end-stage hepatic diseases. Nowadays, liver transplantation is developing rapidly as a result of the perfect perioperative treatments and widespread applications of some immunosuppressants. However, there is an obvious problem that recipients are greatly more than donors. The disparity between the increasing demand for liver donors and the limited supply of donor organs has led to a reconsideration of the use of marginal pools, such as NHBD[25-27]. NHBD programs have been used successfully in kidney transplantation, and were able to increase donor pool of kidneys by 40%. In the field of liver transplantation the use of livers from NHBD shows a less favorable clinical results, because of the limited hepatic tolerance against warm ischemic damage compared with kidneys[28-30]. Although several clinical and experimental studies have shown that liver can compensate 60 min of WIT during liver resection[31,32], in transplantation settings, there were few studies about limit of the period of warm ischemia before transplantation. To evaluate the quality of liver grafts and to ascertain the safety time limit for warm ischemia of liver grafts, we should focus on the long-term survival of liver graft and the complications after transplantation[33].

In present study, we found that the pathologic changes of hepatic cells undergoing only warm ischemia injury was irreversible when WIT was less than 60 min, only part of hepatic cells underwent irreversible injury. But the damage to liver graft would aggravate in the cold preservation, operative ischemia stage and the reperfusion process. Histological and subcellular structure changes were reversible in groups with WIT less than 30 min, especially at 48 h after transplantation. In WIT 45 min and 60 min groups, hepatocellular degeneration became severer, necrotic cells spread from the center lobule area to a sheet or foci-like area involved several hepatic lobules, the liver graft then underwent irreversible injury[34].

The present study indicates that hepatic injury is reversible within 30 min of warm ischemia injury by cytochemical and histochemical dynamic observation. The glycogen and enzyme-histochemistry activities of liver graft and their recovery potency after reperfusion may serve as criteria to evaluate the quality of liver graft.

ATP, TAN and EC decreased after warm ischemia injury, especially in the first 30 min, the change was reversible within 30 min of warm ischemia injury. WIT and hepatic energy metabolism recovering potency were key factors to the postoperative survival. ATP, EC and their recovering potency of the liver graft may serve as criteria to evaluate the quality of liver graft.

Microcirculation of grafted liver could be gradually resumed normal after reperfusion when WIT was less than 30 min, which indicated that hepatic cells held the recovery potency and could regained normal microcirculatory structure after reperfusion if

the WIT was less than 30 min. After 45 min of warm ischemia, most hepatic sinus was unobstructed, but there were still some sinus filled with cytoplasm blebs, reticular fibrosis and hemocytes. So 45 min may be the deadline of hepatic warm ischemia. When WIT was more than 60 min, microcirculatory structure of liver graft presented irreversible injuries.

Besides the researches on the influence of warm ischemia time on energy metabolism, morphology and function, special attention was paid on the influence of warm ischemia on the postoperative long-term survival and complications. Correspondence analysis showed that complications in groups with WIT less than 30 min were similar; and the incidence of liver dysfunction increased gradually in a time-dependent manner; incidence of biliary obstruction increased with the elongation of survival time of the recipient rats. Kaplan-Meier analysis showed that survival time in groups with WIT less than 30 min had no significant difference. In WIT 45 min group, the animal might still survive early postoperative period, but the long-term survival was severely insulted, when WIT prolonged to 60 min, both function and histological structure of graft would develop irreversible damage and no recipients could survive.

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