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Can the rat donor liver tolerate prolonged warm ischemia ?

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INTRODUCTION

The last two decades of the twentieth century have witnessed increasingly successful rates of liver transplantation. The number of liver transplantations has increased steadily while the number of organ donors has remained relatively constant. Thus a great disparity has developed between the demand and supply of donor organs and remains a major limiting factor for further expansion of liver transplantation. Although many procedures, such as split liver[1], living-related transplantation[2], and xenotransplanta tion[3], have been attempted clinically to overcome the shortage, it is hoped that livers harvested from non-heart-beating donors (NHBDs) would alleviate the problem of organ shortage, which again becomes the focus of attention[4-9]. However, sensitivity of the liver to warm ischemia remains a major worry for use of the NHBDs. The aim of this animal study was to assess if murine liver could tolera te prolonged period of warm ischemia and to determine the optimum timing of inte rvention in the cadaver donor in order to preserve liver viability.

MATERIALS AND METHODS

Animals

Male Sprague-Dawleyrats, weighing 180 to 220 g, were obtained from Sh anghai BK Lab Animals Co. Ltd. Donor and recipient rats were matched for size. R ats were housed in the standard animal room without fast before the surgery and with free activity and standard diet after surgery.

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Experimental design

Orthotopic liver transplantation was carried out in 5 groups with non-heart-beating time in the donor ranging from zero to 15, 30, 45 and 60 min respectively (Group 1-5). The period of non-heart-beating was counte d from the donors cardiac arrest to the beginning of the cold perfusion of the liver. Each group consisted of 13 rats; 5 rats were sacrificed one hour after surgery for sample collection and graft biopsies; the other 8 rats were used to observe one-week survival rate; and rats that survived for more than 7 d after transplantation were considered as survivors. Except for 1 or 2 survivors that were randomly selected to observe the possibility of long-term survival, the others were also sacrificed 7 d after surgery.

Animal surgery

Both donor and recipient surgery was performed at room temperature of 21℃ to 22℃ under ether anesthesia. No immunosuppressive agents were given to either donor or recipient. Five min after the intravenous administration of 500 IU o f hep arin, donor cardiac arrest was induced by opening the chest and cross clamping t he base of the heart with hemostats. Donor surgery began 15 min prior to the end of the designated warm ischemic time in each group. All livers were perfused with 10 mL of 4℃ Ringer's lactate solution through the abdominal aorta, and stored in the same cold solution for an average cold ischemia of 60 min. The don or liver was implanted to the recipient according to the cuff technique of Kamad a and Calne [10] with an average anhepatic phase of 16 min. No heparin was administrated to the recipientrat.

Sample collection and analysis

The blood samples were obtained one hour and seven days after hepatic implantation for measurement of serum ALT and AST, meanwhile liver tissues were also taken for histologic examination. Furthermore, one hour after reperfusion serum TNF level was detected by the bioassay using L-929 cell lines as described elsewhere. The graft tissues were fixed in 10% formaldeh yde, and sections were stained with hematoxylin and eosin.

Statistics

Values are expressed as the $\overline{x} \pm s$. Statistical significance was c ompared with group 1 and tested by unpaired Student's *t* test. The survival rates in each group were compared with group 1 using Fisher's exact test. *P* values less than 0.05 were considered significant.

RESULTS

Survival

No recipient death was directly related to operative echnical failur e in this study. The one-week survival rate and the long-term survival period of rats that underwent liver transplantation are listed in Table 1. With a stepw ise increase of the non-heart-beating period from zero to 15, 30, 45, and 60 min in donors, the survival rates in recipients were 100, 75, 62.5, 25, and 0 percent respectively. Moreover, rats that received grafts having suffered 15, 30, or 45 min warm ischemia before implantation showed the possibility to su rvive more than 60 d. The longest time for using NHBDs in our study was 45 min. A total of 19 recipient rats died within 7 d, among which 15 deaths were caused by primary graft nonfunction, with massive ascites and patchy liver ne crosis at autopsy. Two rats died of biliary complications and 2 died of pulmon ary infection. The survivor in Group 3 also died of pulmonary infection 18 d after surgery.

Table 1 Survival status

Group	Warm ischemia / min	One-week survival rate	Long-term survival / day
1	0	100% (8 / 8)	>60, >60
2	15	75% (6 / 8)	>60, >60
3	30	62.5% (5 / 8)	18, >60
4	45	25% (2 / 8)[a]	>60
5	60	0% (0 / 8)[a]	

[a]$P<0.01$ *vs* Group 1

Hepatic function

The mean serum ALT and AST concentration one hour after reperf usion was significantly higher in Groups 2, 3, 4, 5 compared with Group 1, and the concentration of enzymes dropped markedly after 7 d. The postoperative increase in enzyme concentration appeared to be proportional to warm ischemia time.

Table 2 ALT and AST values after transplantation

Group	One hour after reperfusion		7 d after operation	
	ALT(u / L)	AST(u / L)	ALT(u / L)	AST(u / L)
1	362 ± 49	623 ± 75	38 ± 9	115 ± 19
2	546 ± 68[a]	873 ± 95[a]	64 ± 11[a,b]	345 ± 66[a,b]
3	1096 ± 195[a]	1435 ± 238[a]	124 ± 35[a,b]	454 ± 120[a,b]
4	1505 ± 326[a]	2001 ± 376[a]	184	732
5	2357 ± 534[a]	2886 ± 590[a]		

[a]$P<0.01$ *vs* Group 1; [b]$P<0.01$ *vs* 1 h after RPF

Serum TNF

The mean serum TNF levels one hour after reperfusion in Group 1 to 5 were (1.84 ± 0.21) U/mL, (2.62 ± 0.29) U/mL, (4.0 ± 0.4) U/mL, (4.5 ± 0.4) U/mL and (7.4 ± 0.6) U /mL respectively. The degree of serum TNF elevation was associated with the severity of warm ischemia injury to donor liver.

Histological examination

One hour after reperfusion, the livers whether or not subjected to warm ischemia all showed basically well-preserved hepatic architecture without lymphocyte in filtration in the portal area. In Group 5, mild diffused hepatocyte vacuolizatio n was identified, accompanied by dilatation of the sinusoids. One week after transplantation, the livers of the different groups manifested lymphocyte infiltrat ion in the portal area and congestion in the sinusoids. Neutrophil infiltration in the portal area was also identified in Groups 3 and 4. Furthermore, mild lobu lar necrosis surrounding hepatic vein was found in Group 3; moderate lobular nec rosis and ballooning degeneration were found in Group 4.

DISCUSSION

Ever since the guidelines for defining brain death have been established, brain dead donors with beating hearts have been the commonest source of transplant organs[8]. However, the concept of utilizing organ allograft from non-heart-beating donors for transplantation is not new. During the early stage of tran splantation, NHBDs were the only source of unpaired allograft, i. e. hearts and livers. With refinements of surgical techniques, improvements in immunosuppressi on and development of effective organ preservation, the increasing success of and widening indication for liver transplantation has exacerbated the shortage of donor organs, and resulted in an increasing number of potential allograft recipi ents dying on waiting list. To increase the number of livers available for liver transplantation, grafts procurement from NHBDs has again become the focus of at tention. Furthermore, recent estimates indicate that an increase of 20% to 25% i n organ donors could be realized if NHBDs were used routinely[12].

During the past two decades, the belief in the extremely high sensitivity of hepatic parenchyma cells damage from warm ischemic injury has been challenged. In both clinical and experimental studies, it has been suggested that the live r can tolerate warm ischemia up to 60 min[13-18]. Warm ischemia of graft is considered a risk factor for postoperative graft dysfunction after liver transplantation. However, in liver transplantation from NHBDs, the effect of warm ischemic damage is subsequently compounded by preservation (cold ischemia) a nd reperfusion injuries. The tolerance of the liver to warm ischemia in such situations is still controversial.

Generally liver allografts can be preserved at tow temperatures safely for up to 24 h in University of Wisconsin solution. In transplantation setting, howe ver, there are a few studies about the limit of the period of warm ischemia before transplantation. In rodents, Soejima *et al*[19]reported that none of their control rats exposed to 30 min warm ischemia before transplant ation survived more than 2 d, and also the similar result was seen in Sumimot o's study[20]. However, Ikeda[21]reported that all

the rats suffering 30 min warm ischemia before transplantation survived more than 3 wk . Moreover, in 1995 Xu and Jones reported 50 per cent of one-week survival rate after 100 min of non-heart-beating time[22], and also Takada re ported his successful pig liver transplantation from NHBDs exposed to 60 min warm ischemia[23]. Our data were compatible with Ikeda's[21] and Richter's[24]. The present study unexpectedly found that rat livers harvested from NHBDs after 30 min of cardiac arrest could be successfully gr afted to the recipients with a 62.5% one-week survival rate and hav e the possibility to survive more than 60 d. No statistical significance of one-week survival rates was found between Group 1 and 3. In Group 4, even the don or liver suffered 45 min of warm ischemia, two of eight (25%) rats s urvived more than a week, also with a chance of long-term survival. The results confirmed that the liver is less sensitive to warm ischemia than formerly belie ved.

Hepatic ischemia-reperfusion injury is characterized by sinusoidal perfusion failure, accumulation and adherence of leukocyte in sinusoids, loss of the end othelial integrity with concomitant extravasation of blood cells, and, finally, breakdown of the liver microcirculation[25]. Under inflammatory conditi ons, there are several factors that would favor neutrophil trapping. First swell ing of Kupffer cells and endothelial cells occurs during exposure to inflammatory mediators. Second, an imbalance of vasoconstrictor (e.g. endothelin-1) and vasodilator (e. g. nitric oxide) formation can further narrow the sinusoidal diameter[26,27]. Moreover, inflammatory mediators such as activated complemen t factors reduce the deformability of neutrophil. The combination of these factors appears to be responsible for the microcirculation failure.

It is widely accepted that warm ischemia and cold ischemia represent two differe nt and distinct types of injury to the liver. While cold ischemia mainly causes injury to the cells of the sinusoidal lining, hepatocytes are the most vulnerable to warm ischemia[21,28,29]. The postoperative elevations in the serum liver enzyme concentration observed in the present study may reflect the extent of hepatocyte disruption, which was more severe in proportion to the duration of warm ischemia. TNF procuded by Kupffer cells plays an important role in the pathogenesis of early graft failure[30-33]. In this study, we demonstrated an elevation of serum TNF levels in association with increasing prolonged wa rm ischemia time. The degree of histological damage and serum ALT and AST value changes correlated positively with the TNF levels.

In conclusion, rat liver can tolerate a prolonged warm ischemia time up to 45 min for transplantation with a chance of recipient survival. Based on our experience, warm ischemia is not the only factor limiting the success of liver transplantation from NHBDs[34]. More attention should be paid to other factors such as the mechanical injury during the harvest of cadaver donor liver and to the cold preserve time and anhepatic phase. Actually, clinical experience with the use of liver allograft from NHBDs has been reported[4,5,35]. The patients, who had irreversible neurologic damage but did not meet standard brain death criteria, were transported to the operating room where ventilation w as discontinued, and rapid cold perfusion was performed after declaration of death. In such controlled circumstances, the warm ischemia time was less than 30 min in most cases. Some of the procured grafts were actually transplanted, exh ibiting satisfactory initial function. However, in the patients who suffered a sudden cardiac arrest, in whom cardiopulmonary resuscitation was necessary before organ procurement (referred to as uncontrolled NHBDs), there was a high inciden ce of primary graft nonfunction. In this regard, the present study is a simulati on of well-controlled NHBDs, and further intensive experimental studies are required to approach this goal, because human NHBDs have much more complicated clin ical conditions than healthy rat donor. In clinical liver transplantation, unsta ble hemodynamic conditions before cardiac arrest may produce a warm ischemia injury. New strategies to attenuate ischemic liver injuries will be required to est ablish the safety and efficacy of liver transplantation from human NHBDs.

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