The simple maneuver to safely extend the anhepatic time to 30 minutes for rat liver transplantation

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Abstract

Background

Orthotopic rat liver transplantation (OLT) is widely used; it remains to be challenging although many renovations have been made.

Methods

For tolerance induction, we performed OLT including whole graft OLT from close Sprague Dawley (SD) rat to SD (whole graft group, control group, n = 21) and 30 minute anhepatic time (AHT) group (AHT group, n = 11). For good outcomes of AHT group and generalization of this maneuver, partial OLT was performed from 50% Lewis allograft to Brown Norway (BN) rats to induce tolerance (half graft group, n = 28), Cyclosporine A was injected once daily for 14 days.

Results

For whole graft group, 30-day survival rate was 85.5% (18/21), the reasons of death were gas embolism due to the missed suturing in 2 cases, blood loss in 1 case. For AHT group and tolerance group, 30-day survival rate was 72.7% (8/11), 64.3% (18/28), the causes of death were diverse. There were no differences in survival in 3 groups ($p = 0.289$).

Conclusion

The anhepatic time can be extended simply through the change of clamping the diaphragm, which facilitates its application in the research.

Background

Rat OLT is well-accepted model especially in the research of tolerance induction since Lee and his colleagues developed [1,2], it is complicated in microsurgical techniques, especially for new microsurgeons although many valuable modifications have come into forth [3–5]. It is documented that the short anhepatic time plays a critical role of the success of LT and improves survival greatly, and that cardiac arrest in the recipient frequently occurred when AHT was more than 26 minutes [6,7]. Higher mortality rate during AHT necessitates researchers to extend AHT. Here we first introduce the simplest method to extend AHT to 30 minutes while facilitating this complicated procedure.

Methods

For our project, rat OLT was performed to study liver regeneration and immunological tolerance through stem cells (detailed protocol out of scope here).Following the procedure in the literature, we observed that clamping the diaphragm led to abnormal breathing and rat death (supplemental video 1), and that the change of clamping the diaphragm benefited this microsurgical procedure and improved rat survival. Whole OLT was performed from close SD to SD with different AHT, and partial OLT was performed with Lewis to BN as the acute rejection model whereas this technique was generalized and applied in our project (Table 1).

Table 1
data for recipients of 3 groups. other*for unknown reasons. AHT, 30- minute anhepatic time

Anhepatic time	30-day survival rate	Death reasons
whole graft (n = 21)	≤ 20 min 85.5% (18/21)	missed suture(2),bleed(1)
AHT group (n = 11)	30 min 72.7% (8/11)	respiratory failure(1),other*(2)
half graft (n = 28)	≤ 20 min 64.3%(18/28)	diarrhea(4),wound(6)

Animal

Male rats including close SD, Lewis (weighing 200-400g) and BN were used as donors and recipients purchased from Beijing Vital River Laboratory Animal Technology Corporation. The animals were housed with controlled temperature and light, freely access to a standard chow diet and water; they were fasted 12 hours prior to the operation.

All experiments were approved by the ethics committee of Henan Provincial People's Hospital and conducted in compliance with the standards for animal use and care set by ARRIVE guidelines and the Institutional Animal Care Committee of Henan Provincial People's Hospital.

Surgical Procedure

Isoflurane inhalation anesthesia was applied with a few modifications. In the donor procedure, a transverse incision was made to enter the abdominal cavity of the rat. the liver was flushed through the aorta with lactated Ringer's solution, and then reflushed through the portal vein (PV). The graft was immersed in lactated Ringer's solution. Cuffs were prepared for PV and infra-hepatic vena cava (IVC). For 50% graft, the caudate lobes, the left lateral one and the left portion of the median lobe were removed at the back table. Cold storage time was less than 3 hours in all cases. In the recipient, after the abdominal cavity was opened like the donor, all ligaments of the liver were cut. The proper liver artery was ligated proximally, and the accessory liver artery was ligated and cut, a blunt separation behind the liver was made to create a tunnel. The left sub-diaphragmatic vein was ligated closed to the diaphragm. The recipient PV and IVC were clamped with microvascular clamps, and isoflurane was immediately decreased to 0.3 volume %. A mosquito forceps was placed through the tunnel on the part of diaphragm

ring (left side) to occlude SHVC and stabilized (Fig. 1), SHVC was anastomosed with 8 – 0 polypropylene running suture (Fig. 2), when this anastomosis was completed, the forceps was replaced with a vascular bulldog on the real SHVC while the diaphragm ring was de-clamped (Fig. 3), the anhepatic time was generally less than 20 minutes. PV was reconnected with the cuff; blood flow was restored once the clamp on the PV was released. IVC reconnection was made as was for PV. For AHT group, the clamp on the PV was released until 30 minutes. The gastroduodenal artery was proximally ligated and an opening was made on the common hepatic artery into which the stent in the donor hepatic artery was inserted and secured (Fig. 4, supplemental video 2), bile duct continuity was made when a tube in the donor bile duct was inserted into the recipient bile duct. The abdomen was closed with two layers and the animals were kept in a cage under an infrared light. 10% glucose solution and purified water were both supplied for first 3 days, and later regular food and tap water were offered. Sodium ceftizoxime (100mg/kg) was injected subcutaneously once a day, 4 days in total. For half graft group, cyclosporine A was subcutaneously injected once daily for 14 days and ceased afterwards. Sacrifice was made on deep anesthesia with isoflurane for euthanasia.

Statistical analysis

The cumulative survival rates of different groups was evaluated with the Kaplan-Meier Curve, the analysis was made with SPSS 22.0 software (IBM Corp, Armonk, NY, USA), and $P < 0.05$ was considered significant

Results

For the whole graft, 30-day survival rate was 85.5% (18/21), the reasons of death were gas embolism due to missed suturing during the anastomosis in 2 cases, blood loss in 1 case. For 30-min group, 30-day survival rate 72.7% (8/11), the causes of death were respiratory failure in one case; unknown reasons were in 2 cases. For half graft group, 30-day survival rate was 64.3% (18/28) (Fig. 5), the reasons of death were diarrhea in 4 cases, BN rats ate themselves in 6 cases. There were no differences for survival for 3 groups($p = 0.289$).Histological examination revealed almost normal structures of liver without fibrosis, ductopenia, thickened wall in the liver arterioles and venules in half graft group (H&E staining not shown).

Discussion

Since Kamada introduced the cuff method for OLT, it has greatly benefited OLT in basic research [1, 2, 6]. Short AHT as a surgical skill has been prioritized to minimize as soon as possibly by clinicians and microsurgeons who perform liver transplantation. Clinically it ranged from 37 to 321 minutes, it was reported that over 100-minute AHT was associated with a higher incidence of graft dysfunction [6,8]. Experimentally, the AHT ceiling of rat OLT is 26 minutes in the literature [1,6]. For our report, it is safely extended to 30 minutes (even 35 minutes) whereas survival rates were not significantly different, in the time-efficient manner (30 min), the different anhepatic time has little impact on recipients survival.

Continuous suture and cuff method comprise the reconstruction of SHVC [1.2.9–14], suturing should be completed rapidly while SHVC is blocked and it necessitates the diaphragmatic ring being clamped a little more without ventilator aid. The diaphragm is to maintain respiratory movement, once clamped and retracted downward (caudalad), it dramatically affects respiratory and causes the rat to move due to compromised respiration (supplemental video 1), some surgeons might add anesthesia, resultantly the rat will die, especially under plain and simple mask anesthesia inhalation (ether etc), this is the reason that cardiac arrest and higher mortality occurred during AHT [7]. Cuff method efficiently shortens SHVC anastomosis, but it is not universally applicable due to short SHVC in length. Magnetic ring is a cuff method in nature and precludes future MRI examination [13,14].

Pharmaceutics extension AHT came forth in some centers, prostaglandin and its analogue were used to extend AHT and improve survival [15,16]. Liu et al reported that clamping the supra-celiac aorta one minute can effectively improve rat OLT by increasing the tolerable time of AHT [6], this maneuver does not surpass the AHT ceiling. Our maneuver is surgically easy and effectively extends AHT to 30 minutes. A few references were reviewed to reveal that the diaphragm was clamped much more [17,18], we followed that procedure which led to irregular breathing (supplemental video 1). In the literature almost no attention has been paid to how to clamp SHVC or the diaphragm on the reconnection of SHVC, our report is the first description of clamping the diaphragm. As to tolerance induction in our report, it is ongoing research of our project and the protocol is out of scope here.

Conclusion

OLT can be safely performed with 30 minute AHT extended simply through the change of clamping the diaphragm; this procedure facilitates its application in the research.

Declarations

Ethics approval and consent to participate

All experiments were approved by the ethics committee of Henan Provincial People's Hospital, and conducted in compliance with the standards for animal use and care set by ARRIVE guidelines and the Institutional Animal Care Committee of Henan Provincial People's Hospital.

Consent for publication

N/A

Availability of data and materials

All data and materials are available on request□ST Zhou is responsible for all data.

Competing interests

The authors have no conflicts of interest to declare

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Authors' contributions

GF.Tang wrote the draft. JB.Zhang discussed and revised the draft. HB.Zhao collected data and caring.SD. Wei performed the statistical analysis. ST Zhou performed OLT, conceived, designed and finalized the study. GY. Chen funded the study. All authors have read and approved the manuscript.

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Abbreviations

orthotopic liver transplantation, OLT; anhepatic time, AHT; Sprague Dawley, SD; Brown Norway, BN; portal vein, PV; supra-hepatic vena cava, SHVC;

infra-hepatic vena cava, IVC

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Table

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AHT group (n=11)	30 min	72.7% (8/11)		respiratory failure(1),other*
half graft (n=28)	(2)			
	≤20 min	64.3%(18/28)		diarrhea(4),wound(6)