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EXPERIMENTAL MODELS IN LIVER TRANSPLANTATION SURGERY: A SHORT REVIEW

MODELE EXPERIMENTALE ÎN TRANSPLANTUL HEPATIC: O SCURTĂ RECENZIE

H. BOCȘE[1)], A. BARTOȘ[1),2),*)], Ioana IANCU[1),2)], C. POPA[1),2)], S. BRÎNZILĂ[1)], A. HERDEAN[1),2)], F. GRAUR[1),2)], E. MOIȘ[1),2)], Dana BARTOȘ[1),2)], F. ZAHARIE[1),2)], Daniela IONESCU[1),2)], C. IANCU[1),2)], C. PUIA[1),2)], N. Al HAJJAR[1),2)]

ABSTRACT | REZUMAT

End-stage liver disease is currently a social and economic burden worldwide. Liver transplantation (LT) is the only curative treatment for patients with this condition. High volume centers and intensive surgical training are mandatory for optimal results of this procedure due to the current shortage of donors. Experimental models have been proposed for more than 60 years ago but the continuous development of surgical techniques emphasizes the need of adjustments. The review aims to present up-to-date aspects of LT experimental models both with advantages and pitfalls of the surgical procedures.

Keywords: liver transplantation, experimental model, animal model, experimental liver surgery

Boala hepatică în stadiul terminal este actualmente o povară la nivel global din punct de vedere economic și social. Transplantul hepatic (TH) este singurul tratament curativ pentru acest stadiu al bolii. Centrele cu volum mare al intervențiilor și cu training continuu al echipelor chirurgicale reprezintă două caracteristici esențiale pentru obținerea unor rezultate optime, în condițiile scăderii numărului de donatori. Modele experimentale pentru TH au fost propuse în urmă cu peste 60 de ani, însă dezvoltarea tehnicilor chirurgicale susține faptul că este nevoie de îmbunătățiri ale rezultatelor. Scopul acestei recenzii este de a prezenta date de actualitate în modele experimentale ale TH cu avantajele și dezavantajele fiecărui model propus.

Cuvinte cheie: transplant hepatic, modele experimentale, modele animale, chirurgie hepatică experimentală

Liver transplantation (LT) is currently the only effective treatment for end-stage liver disease. Since the first human liver transplantation (HLT) was performed by Thomas Starzl in 1963, many researchers focused on technical aspects, immunosuppressive therapy, donor selection and prophylaxis of infections (32). Hence, nowadays many conditions associated with end-stage liver disease are referred to LT.

Despite almost 60 years of extensive experience in HLT, there is an increased number of patients waiting for LT and a shortage of donors that has become a real burden for the healthcare system. Thus, extensive experimental research is still needed in order to improve this field, especially for better identifying all potential donors and to avoid unnecessary graft loss (17).

Since 1952, when the first LT was performed on canine species (5),different experimental models have been proposed. In mice models, liver allografts are accepted indefinitely across major histocompatibility complex barriers, consequently anatomical, and surgical aspects define the subject selection (4). The availability of genetically modified mice provides a great tool in terms of immunosuppressive therapy compared to rats and pigs. However, the microsurgical techniques necessary for these models still represents a pitfall. The first experimental procedures consisted of removing the native liver with the complete resection and reconstruction of the retrohepatic vena cava (13). Nowadays, the vena cava preserving technique, also known as piggy-back technique is the most widely used. These techniques require high surgical skills. The importance of performing liver transplant in high volume centers has already been highlighted as patients undergoing liver transplant in low-volume hospitals have up to 30% higher odds of death at 1 year compared to high-volume centers (1).

The review relates by a narrative approach, the current knowledge of experimental models in liver transplantation and how they can be implemented for research in terms of surgical technique and learning curves. Hence, surgical teams that seek to start or im-

1) Regional Institute of Gastroenterology-Hepatology, Cluj-Napoca, Romania
2) University of Medicine and Pharmacy "Iuliu Haţieganu", Cluj-Napoca, Romania
*) *Coresponding author: bartos.adi@gmail.com*

prove LT techniques can find an up-to-date information that can lead to a shortened learning curve.

METHOD OF RESEARCH

Systematic research was carried out in Pubmed, Scopus, Embase using the keywords "liver transplantation", "experimental model", "animal model", "experimental liver surgery" for papers published in English from 2006 to September 2021. All identified papers were reviewed by abstracts and the identified studies were included for a narrative review. References from selected articles were also included. The primary endpoint was to present the current experimental models used in LT and the secondary to address the issue of the learning curves for each model.

ORTHOTOPIC LIVER TRANSPLANTATION IN MOUSE MODEL (MOLT)

Mice are an ideal model for research due to a well characterized genome and a great availability of genetically modified models. In surgical research, the anatomical similarities between mice and humans provided a great tool for complex procedures such as LT (24). Moreover, there are similarities between H-2 system in mouse and HLA human complex, which is an important factor in transplant models (8).

The first mouse orthotopic liver transplant (MOLT) was described by Qian et al. in 1991 (31) and since then, different techniques and instruments have been proposed in order to develop an ideal model (23, 38).

The pitfall of mouse model in liver transplantation is represented by a steep learning curve due to the demanding surgical technique that requires months of training.

Animal selection

The burden of major histocompatibility complex barriers is not an issue in mice models, thus only the surgical and anatomical aspects are defining the selection (4). Comparing to rat model, the liver vessels are on average eight times smaller in mouse. Literature data shows the preference of male models due to a larger body size (24). Mouse weights between 23-33g are optimal for OLT in terms of surgical technique (38, 40).

Anaesthesia

Standard inhalational anaesthesia is represented by isoflurane in MOLT as most injectable anaesthetics are metabolized in the liver which can eventually alter hepatic function. Inhalational anaesthesia is characterized by low hepatotoxicity, rapid excretion and can prevent jeopardizing cardiopulmonary function (7, 21). Targeted isoflurane concentration should be 3-4% in the induction phase, 2% in the maintenance phase, and <0.5% in the anhepatic phase. Injectable drugs combination has been described by using ketamine-xylazine-acepromazine with no side effects on liver graft or 7-day survival (14).

Organ procurement

The key points of the operative technique are: development of sufficient surgical skills to minimize liver trauma, minimum liver ischemia time and secured anastomosis of hepatic vessels and bile ducts (31, 39). A wide abdominal incision is needed to expose the liver. The liver should be freed from ligamentous attachments. The intrahepatic inferior vena cava (IHIC) and the portal vein (PV) need to be dissected to the level of the left renal vein and the superior mesenteric vein to ensure an optimal length of cuff for the anastomosis (24). For hepatic artery (HA) reconstruction, several reconstruction models have been proposed: HA ligation for nonarterialized model, preparation of hepatic–celiac–aortic arterial segment or hepatic–celiac–aortic–mesenteric arterial segment for suturing anastomosis (15, 36). For bile duct stenting polyethylene tube, Venflon tube or epidural catheter can be used (15, 30). Heparinization, biliary cannulation and perfusion are the most important steps in organ procurement. Perfusion is performed to flush out the donor's residual blood and to rapidly cool the liver (24).

Back-table preparation

During this step, the graft is prepared for transplantation. The donor liver should be stored in a cold preservation solution 4C (24). Nowadays, most of the surgeons are using the standardized two-cuff technique that ensures the eversion of the inner endothelial surface over the cuff material(polyethylene) which eventually facilitates the PV and IHIC anastomosis (3, 33).

Implantation surgery

The particularities of this step are represented by the dissection of IHIC and PV to the level of the right renal vein and pyloric vein followed by anastomosis and the arterial reconstruction. In nonarterialized model, the hepatic artery is ligated and divided. In arterialized model, the common hepatic artery is prepared for stent insertion or an infrarenal aortic segment is prepared for an end-to-side anastomosis.

The SHIVC is reconstructed with a 10-0 nylon suture anastomosis. Reconstruction of the bile duct is performed by inserting the donor stent in the recipient's bile duct (24).

Anhepatic time is a decisive prognostic factor for MOLT and a period of maximum 25.8 +/- 3min for arterialized model is tolerated (16).

MOLT is a complex model for research but the ad-

vanced surgical skills for the procedure allow only few centers and research groups to perform the procedure.

ORTHOTOPIC LIVER TRANSPLANTATION IN RAT MODEL (ROLT)

The first liver transplantation in rat model was published by Kamada et al. in 1979 (19). Results from Oldani et al. (2012) showed mastering of technique after 10 procedures (29). The surgical technique described by Oldani et al. (2012) is similar to the standard two-cuffs technique. In 2005, Kashfi et. al (2005) described in a review nearly 30 techniques used for ROLT (22).

Reconstruction methods

SHIVC: microsuturing technique (end-to-end 7-0 suture), cuff technique (donor cuffed SHIVC inserted in recipients SHIVC fixed with 3-0 suture) (22).

IHICV: microsuturing temporary splint technique (anterior wall sutured over a temporary splint followed by a 180 degrees rotation for posterior wall suture), microsuturing technique (end-to-end anastomosis 8-0 suture), cuff technique (donor cuffed IHIVC inserted in recipients' IHIVC; fixed with 6-0 suture) (22).

PV: microsuturing temporary splint technique (same technique described for IHICV), micro suture technique (end-to-end 8-0/9-0 suture), cuff technique (PV in-serted in recipients' PV; fixed with 7-0 suture) (22, 25).

HA: microsuturing technique (Aortic segment: end -to-side anastomosis, Celiac segment-end-to-end anastomosis with right renal artery after nephrectomy), splint technique (thin teflon catheter inserted in the donor's HA; the splint is introduced in the recipient's HA and fixed with 2 stitches), cuff technique (a cuff attached to the recipient's right renal artery is inserted into the donor aortic segment), sleeve technique (the recipient HA is inserted into the lumen of the donor common HA), telescopic technique (a silastic tube is entered into the donor celiac artery and a polyethylene tube is inserted into the recipient's HA; the recipient's cannulated artery is telescoped into the donor HA), no arterialization (20, 22).

Bile duct: pull-through technique (the bile duct is cut close to the duodenum in donor and inserted into the recipient's duodenum), telescopic technique (a nylon tube is inserted into the lumen of the BD and another larger tube is inserted into the recipient's BD after an incision into the anterior wall; the donor tube is telescoped into the recipient tube), splint technique (a teflon catheter inserted into the donor BD; at the time of implantation, the splint is introduced into the recipient BD), T-tube insertion (the short arms of the mini T-tube are inserted into the donor and recipient BD; the long arm is brought subcutaneously after externalization). However, in ROLT the two-cuff technique is the standard choice (22).

Regarding costs, research on small animals as rats and mice require lower budgets. Due to a smaller body size, the MOLT has a steeper learning curve comparing to rats. However, the availability of genetically modified mice (knockout, transgenic) and the acceptance of allografts without immunosuppressive therapy, makes them an attractive research model (8, 24, 31).

ORTHOTOPIC LIVER TRANSPLANTATION IN PORCINE MODEL (POLT)

Porcine liver transplantation (POLT) is a standard experimental model due to its similarities with human liver size and anatomy (6, 11, 35). Other advantages of porcine model lie on the absence of hepatic venous sphincter spasm, and low necessity for immunosuppressive therapy after LT (6, 9). General principles of anaesthesia in porcine model have been published in order to provide safe procedures without jeopardizing the experimental results (18).

Organ procurement

The procedure starts with a midline incision from xiphoid process to the pubic symphysis. The exposure of the liver is an important factor in harvesting the liver. Identification of the IHIVC below the liver and cautious dissection to avoid bleeding from the right adrenal vein is the first step of the procedure.

PV dissection should be carefully performed in order to dissect all surrounding tissue and small branches except the superior mesenteric vein (SMV) and the splenic vein (SV). Portal blood flow should be preserved at this point (10). The common bile duct should be isolated and eventually ligated to provide a sufficient length for the anastomosis. The dissection of the HA can be performed to the level of the celiac trunk or with an aortic patch (Carrel patch) (27). Adequate perfusion of the donor graft and cooling down the liver(<12C) are crucial for optimal results.

The SHIVC is harvested with 3-4 cm of diaphragmatic rim for the anastomosis and the donor liver is kept in a cold Ringer lactate solution (18).

Recipient operation

A major pitfall in POLT is poor tolerance of portal venous occlusion and that's why a veno-venos bypass (VVB) is indicated in order to avoid splanchnic congestion. The diversion of the venous flow to the suprahepatic caval system is usually performed (2, 26).

After the hepatectomy is performed and the liver is placed in the right subdiaphragmatic space, the anastomoses are performed in the following order: SHI VC, PV, IHIVC, HA and BD.

Table 1

Advantages and disadvantages of different reconstructions in POLT
(After Esmaeilzadeh et al. 2012)

Reconstructed Structure	Reconstruction Method	Advantages and Disadvantages
Suprahepatic inferior vena cava (SHIVC) Infrahepatic inferior vena cava (IHIVC)	End to end anastomosis Side to side anastomosis (use of prosthesis)	Method of choice Lower risk Simple method Shorter time
Portal Vein (PV)	End to end anastomosis Cuff system PV arterialization	Simplicity Shorter anhepatic time No need for veno-venous bypass Shorter anhepatic time No need for veno-venous bypass Inappropiate for long term survival Simplicity Lower risk of thrombosis Hypotension
Hepatic Artery (HA)	End to end anastomosis Carrel Patch	More effective Simplicity
Bile Duct (BD)	End to end anastomosis Choledochojejunostomy with a Roux-en Y jejunal loop Side to side anastomosis	Simplicity Most physiological Higher rates of bile leakage and stenosis More appropriate for small BD Dysfunction of the Oddi sphincter Needs longer BD Preserves the natural Oddi sphincter

Reconstruction methods

SHIVC: end-to-end anastomosis or using vascular prosthesis. PV: end-to-end anastomosis, cuff technique or arterialization of PV in which donor HA and PV are connected to the suprarenal aorta and the PV is drained into the IHIVC end-to-side. IHIVC: end-to-end anastomosis. HA: using Carrel patch: end-to-side anastomosis to the recipient aorta or end-to-end anastomosis (37). BD: The reconstruction of the BD represents the most delicate step and can be performed with different techniques: end-to-end choledocho-choledochostomy, side-to-side choledocho-choledochostomy, choledocho-jejunostomy with Roux en Y jejunal loop or choledocho-duodenostomy (28, 34).

The larger size of the pig model is an advantage regarding the surgical procedure but for less experienced surgeons it may still represent a challenge due to the complexity of the procedure. Vascular complications(thrombosis) are reported in less than 3% of cases; biliary stenosis or leakage may occur in 9% respectively 4% of cases (12). Appropriate perfusion of the donor liver and a short anhepatic time are two main goals for a safe experimental procedure with positive long-term results (Table 1).

CONCLUSION

The most widely used models in experimental liver transplantation are mouse, rat and porcine models. With more than 60 years of experience, large size animals have the advantage of a shorter learning curve but the advances in immunology, genetics and microsurgical techniques offer new perspectives by using mouse models. The social and economic disparities between countries lead to different needs but the surgical activity of LT should be based on experienced physicians. The need of highly skilled surgeons for performing LT implies standardized training programs and experienced mentors. In order to start LT programs in tertiary centers, experimental models should be mandatory in the training programs due to the delicate aspects involved in the procedure. Fundamental research is an essential aspect of the activity in high volume centers, hence small animal models offer a great opportunity to focus on translational medicine and eventually to deal with the burden of graft loss.

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