—Review—

Surgical Technique of Orthotopic Liver Transplantation in Rats: The Kamada Technique and a New Splint Technique for Hepatic Artery Reconstruction

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Abstract

Orthotopic liver transplantation (OLT) in rats is technically feasible and useful for the assessment of clinical liver transplantation and analysis of inflammatory liver diseases. OLT in rats was pioneered by Lee et al. in 1973 using hand-suture techniques of all vessels. This model has not been widely used due to the long operative time and technical demand. The cuff method was introduced by Kamada in 1979, and today, the Kamada technique is the one most commonly used worldwide. However, this technique does not include hepatic artery reconstruction, although this procedure is routinely performed in clinical transplantation. Nevertheless, several techniques for hepatic artery reconstruction in rat OLT have been reported recently, and our group also developed a simple splint technique from recipient right renal artery to donor celiac axis bearing the hepatic artery. In the present article, we describe the Kamada technique, as a standard surgical method for rat OLT. In addition, we also describe our splint technique for hepatic artery reconstruction. Then, we compare the features of Kamada technique and our splint technique for hepatic artery reconstruction and all other surgical techniques currently in use for rat OLT. The widespread use of the rat OLT model should help to provide full assessment of transplant immunology and the mechanism and treatment of inflammatory liver diseases.
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Key words: animal model, liver transplantation, hepatic artery, rat, rearterialization

Introduction

Liver transplantation is the replacement of a diseased liver with a healthy liver graft. About 50 years have passed since the first clinical liver transplantation by a surgical team led by Starzl in the United States[1,2]. One year after the first liver transplantation, clinical liver transplantation was started in Japan[3]. Liver transplantation from a non-

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heart-beating donor was first performed in 1964 by Nakayama from Chiba University. The first living donor liver transplantation in Japan was performed in November 1989 by Nagasue of Shimane University for a boy with biliary atresia[4]. Makuuchi of Shinshu University succeeded in performing the world's first living donor liver transplantation between an adult donor and an adult recipient in November 1993[5]. After the Japanese government enacted the Organ Transplantation Law in 1997, Kawasaki of Shinshu University performed the first case of liver transplantation from a brain-dead cadaveric donor in Japan in 1999[6]. At Nippon Medical School Hospital, clinical liver transplantation has also been performed by Department of Surgery since 2000[7]. Thanks to advances in surgical techniques and the development of new immunosuppressants, liver transplantation has become an important treatment for hepatic failure.

The advances in liver transplantation are due, in part, to research in small and large experimental animals. Experimental liver transplantation was first attempted about 60 years, at almost the similar period as the start of clinical liver transplantation in the United States[2,8,9]. Since then, new animal models have been developed, surgical techniques have been continuously refined, and experimental liver transplantation is now a versatile research tool that has been used to clarify various issues related to human clinical liver transplantation[10,11]. In particular, the rat has become a suitable model for studying the mechanisms of graft rejection, graft tolerance, and preservation-induced injury in liver transplantation[12–14], based on its technical feasibility, the availability of inbred strains with well-established major histocompatibility complex, low costs, and simple handling. Now, the rat model of liver transplantation has been modified to serve as a model for clinical living donor liver transplantation using partial liver grafts, auxiliary heterotopic liver transplantation, retransplantation using the same liver graft, and xenogeneic liver transplantation[15–19].

Rat Orthotopic Liver Transplantation (OLT) Model

The orthotopic liver transplantation (OLT) technique in the rat was first described by Lee[20,21] and was further enhanced by Kamada by the introduction of the cuff technique[22]. Ishii E. worked with Dr. Kamada at the National Children's Medical Research Center, Tokyo, Japan[23], and learned his technique directly from him. The Kamada technique of rat OLT appears to be well standardized and has become popular, and the model has been used worldwide. The rat OLT model is useful for analysis of clinical liver transplantation. In addition, we consider this model to be important for assessing inflammatory liver diseases. In the present article, we describe the Kamada technique of rat OLT, as a standard surgical technique. Although controversy exists regarding the importance of hepatic artery (HA) reconstruction in rat OLT experiment, we describe our recently developed splint technique for HA reconstruction in rat OLT.

Kamada's Technique for Rat OLT

In rat OLT, at least 4 anastomoses should be performed for donor's and recipient's veins, such as suprahepatic inferior vena cava (SHIVC), infrahepatic inferior vena cava (IHIVC), and portal vein (PV), and the bile duct (BD). In Kamada's technique, the cuff method is used to reconstruct the PV and IHIVC[22]. The anastomosis of the SHIVC is performed with the suture technique, and the telescope method is used to reconstruct the BD.

Animals, Chemical Agents, and Surgical Instruments

Rats 10 to 14 weeks old and weighing 200 to 250 g at the time of surgery were used as both donors and recipients. The rats were housed in Plexiglas cages in a temperature- and humidity-controlled environment and allowed free access to water and normal rat chow. The Animal Studies Committee of our institution approved all experimental protocols

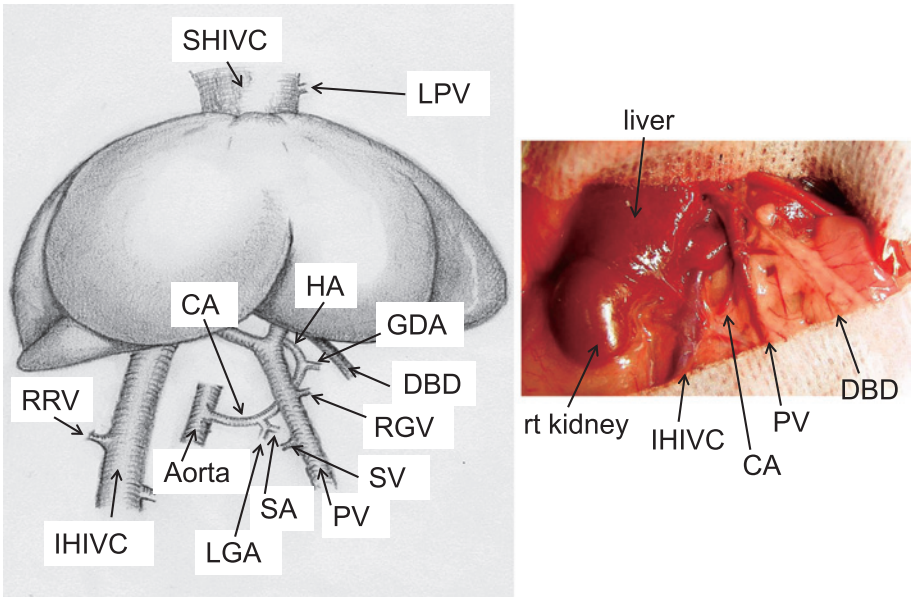

Fig. 1 Donor surgery.
The donor bile duct (BD) is transected, and a telescoping tube is inserted. The suprahepatic inferior vena cava (SHIVC), portal vein (PV), and infrahepatic inferior vena cava (IHIVC) are divided after heparin injection and perfusion of the liver graft using physiologic saline solution. The liver graft is placed in a 4℃ saline bath. CA, celiac artery; DBD, donor bile duct; GAD, gastroduodenal artery; HA, hepatic artery; LGA, left gastric artery; LPV, left phrenic vein; RGV, right gastric vein; RRV, right renal vein; SA, splenic artery; SV, splenic vein; rt kidney, right kidney.

and surgical procedures.

The chemical agents used included heparin (Mochida Pharmaceutical, Tokyo), normal saline (Wako Pure Chemical Industries, Ltd., Osaka), and diethyl ether (Wako Pure Chemical Industries).

Instruments for microsurgery were from Muromachi Kikai Co., Ltd., Tokyo. A 6-0 silk thread was used for cuff preparation, 6-0 proline and 7-0 proline sutures (Ethicon, Inc., Somerville, NJ, USA) were used for anastomosis of the SHIVC, and 3-0 nylon suture on a needle was used for abdominal wall closure. The cuff was made from a 14-G peripheral catheter (14-G Surflo, Terumo Corp., Tokyo) for anastomosis of the PV and the IHIVC. The splint tube for anastomosis of the HA and telescope tubes for anastomosis of the BD were prepared from 22-G peripheral catheter (22-G Surflo, Terumo).

Rat OLT

Rat OLT was performed with the technique of Kamada[22], and the HA reconstruction was performed with our splint technique.

Donor Surgery (Fig. 1)

A transverse abdominal incision is performed under ether anesthesia. The gastrointestinal tract is exteriorized to the left and covered with wet gauze. The PV is divided from the right gastric and splenic veins. The IHIVC is divided from the adrenal vein and the lumbar vein. The right renal vein is ligated and isolated from the IHIVC. The donor BD is transected, and a 0.3-cm-long tube (22-G Surflo) is inserted into the lumen of the BD and circumferentially secured with 6-0 silk suture. Then the gastroduodenal artery, the left gastric artery, and the splenic artery are ligated and divided. Heparin (50 U) is injected intravenously. The celiac axis and the aortic segment are divided. The IHIVC and PV are clamped. The liver is perfused through the PV with an intravenous cannula connected to a syringe containing physiological saline solution. The SHIVC, PV, and IHIVC are divided. The liver graft is placed in a 4℃ saline bath. The donor operation lasts 20 to 30 minutes.

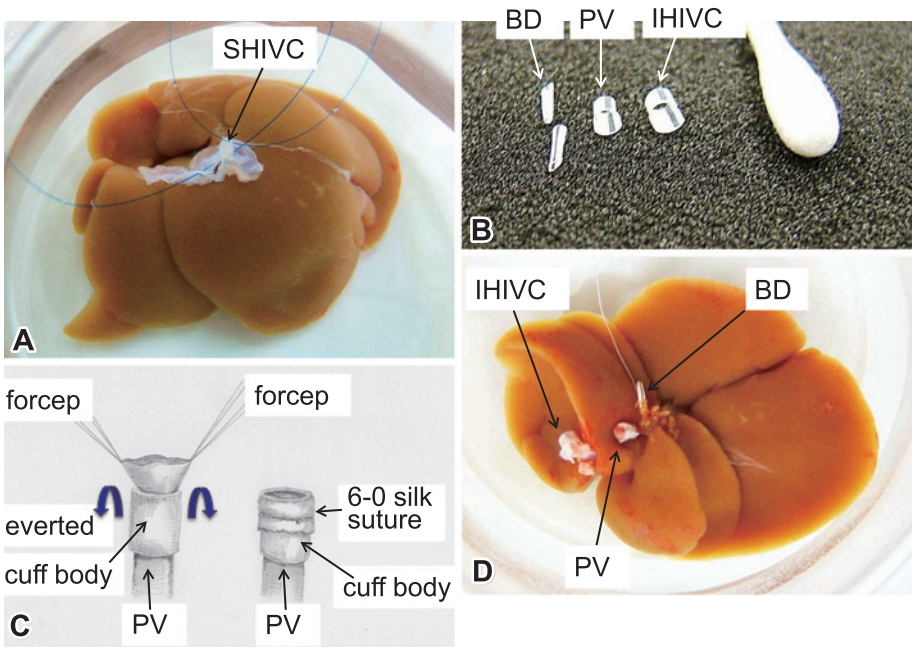

Fig. 2 Liver graft preparation.
A) The liver graft is placed in a 4℃ saline bath. The suprahepatic inferior vena cava (SHIVC) is prepared, and stay sutures are applied using 6-0 proline on both edges of the SHIVC. B) The cuff body with cuff extension for the donor portal vein (PV) and the donor infrahepatic inferior vena cava (IHIVC) are prepared from a 14-G Surflo catheter together with telescoping tubes (22-G Surflo) for the donor and recipient bile ducts (BDs). C) PV cuff attachment; the wall of the PV trunk is completely reversed with microforceps, and the reversed PV wall is fixed on the cuff by ligation of the silk thread. D) In the liver graft, the cuffs are attached in the PV and IHIVC, and the telescoping tube is inserted into the donor BD.

Liver Graft Preparation (Fig. 2)

The cuff segments for the PV and IHIVC are a 0.2-cm-long cuff body with a 0.2-cm cuff extension. The cuff preparation for both vessels is performed in an iced saline bath. The cuff extension is held with forceps, and another forceps is passed through the lumen of the cuff tube to grasp the PV. The cuff is then slipped over the PV. The cuff extension, including the PV, is secured with a bulldog clamp, which is fixed to the wall of the bath container. At this point, the open end of the PV is spread with 2 forceps. The end of the PV is then everted over the cuff body and secured in this position with a circumferential 6-0 silk suture. The same method is used for the IHIVC. To prepare the SHIVC, the diaphragm and connective tissues are completely removed. Stay sutures are then made with 6-0 proline on both edges of the SHIVC.

Recipient Surgery

A midline abdominal incision is made under ether anesthesia. The gastrointestinal tract is covered with wet gauze and kept within the abdominal cavity. The right adrenal and left phrenic veins are ligated and divided. The right renal artery is transected, and a 0.3-cm-long tube (22-G Surflo) is inserted into the arterial lumen and circumferentially secured with 6-0 silk suture. Right nephrectomy is performed after right renal vein ligation. A 0.4-cm-long tube (22-G Surflo) is inserted into the BD and secured with a circumferential 6-0 silk suture. The IHIVC and PV are cross-clamped with microvessel clips, and the SHIVC is cross-clamped with a Satinsky clamp. These vessels are divided, and the recipient liver is removed.

Liver Implantation (Fig. 3)

The donor liver is removed from the iced saline bath and placed in the orthotopic position, and the

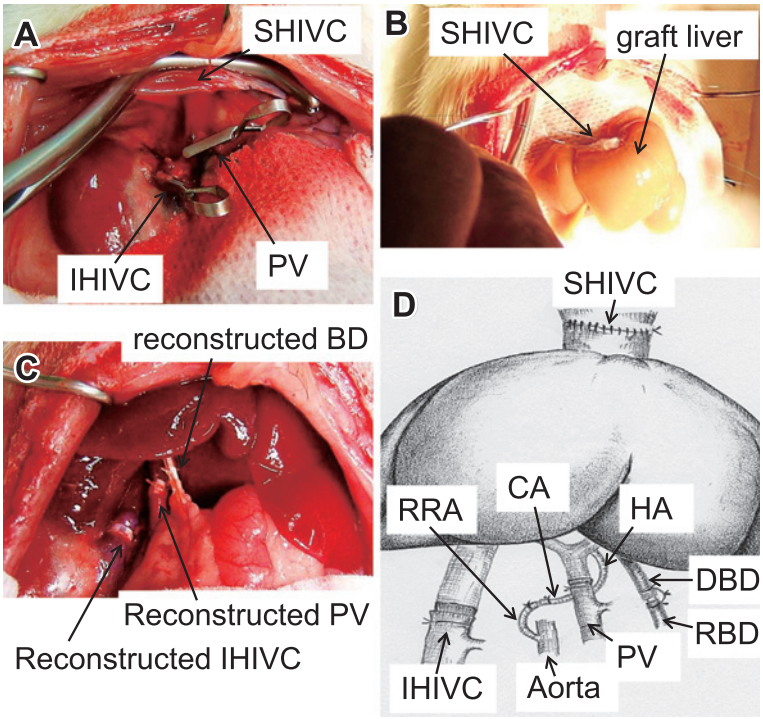

Fig. 3 Kamada technique for rat orthotopic liver transplantation.
A) Removal of the recipient liver, followed by the start of the anhepatic phase. B) The donor liver is placed orthotopically in the recipient rat. C) The donor suprahepatic inferior vena cava (SHIVC) is anastomosed with a running suture. The portal vein (PV) and infrahepatic inferior vena cava (IHIVC) are connected by means of cuff anastomosis. The bile duct (BD) is connected using the telescoping technique. In the figure, anastomosis of the donor and recipient BD, PV, and IHIVC are seen. D) Schematic diagram of the liver graft after the Kamada technique of rat OLT and our splint technique for HA reconstruction. CA, celiac artery, DBD, donor bile duct; HA, hepatic artery; RBD, recipient bile duct; RRA, right renal artery; RBD, recipient bile duct.

donor SHIVC is anastomosed end-to-end to the recipient SHIVC with 7-0 proline. Traction is applied to the distal end of the recipient PV. The recipient PV is irrigated and opened with a needle attached to a syringe containing cold saline. The cuff extension of the donor PV is held with a right-angle forceps. The cuffed donor PV is inserted into the recipient PV. The anastomosis is completed with a circumferential 6-0 silk suture. The clamps on the PV are released, and the anhepatic time is approximately 15 minutes. Next, the IHIVC anastomosis is performed with the same method used for PV anastomosis. The cuffed donor IHIVC is inserted into the recipient IHIVC, and the anastomosis is completed with a circumferential 6-0 silk suture. The BD anastomosis is performed with the telescope technique between the tube secured in the donor BD and the tube secured in the recipient BD. The anastomosis is secured by tying together the ligatures on the donor and recipient BDs (**Fig. 4**). The abdominal incision is closed with a continuous 3-0 nylon suture. The PV clamping time is about 15 minutes, and the IHIVC clamping time is 20 to 25 minutes. The recipient surgery as described in the Kamada technique should be completed within 40 minutes.

Splint Technique of HA Reconstruction in Rat OLT

The HA is reconstructed before BD anastomosis is performed (**Fig. 5**). In the liver graft, the celiac axis bearing the HA is prepared. In the recipient, the right renal artery with a 0.3-cm-long splint tube (22-

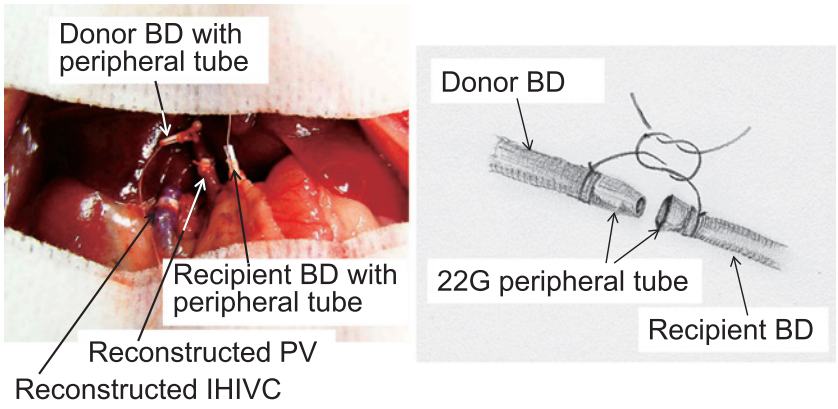

Fig. 4 Telescope technique for bile duct reconstruction.
The bile duct (BD) is connected with the telescope technique. The 22-G peripheral tube of the donor BD is inserted into the 22-G peripheral tube of the recipient BD.

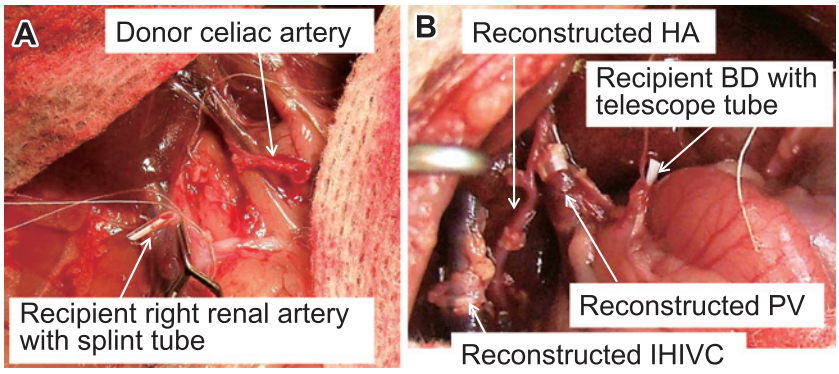

Fig. 5 The splint technique for hepatic artery reconstruction.
A) Hepatic artery (HA) reconstruction is performed between the recipient right renal artery and the donor celiac artery bearing the HA by means of a 22-G Surflo splint tube. B) In the figure, anastomosis of the portal vein (PV), infrahepatic inferior vena cava (IHIVC), and HA are seen. Bile duct (BD) reconstruction is not performed. Note the telescoping tube of the recipient BD.

G Surflo) is prepared as described above. The splint tube secured in the recipient right renal artery is inserted in the donor celiac axis bearing the HA and secured with 6-0 silk suture. In procedures for HA re-arterialization, the connection between the recipient right renal artery with splint tube and the donor celiac artery is completed within 1 minute. In syngeneic OLT between Lewis rats, the patency of the splint anastomosis is confirmed with angiography of the grafted liver through the recipient aorta more than 120 days after surgery **(Fig. 6)**.

Other Surgical Technique of Rat OLT

In rat OLT, several techniques or modifications have been described to improve and facilitate the complex procedures[20－22,24－48]. Each modification represents a change or simplification of the reconstruction methods of 5 anatomical structures (which are the cornerstones of a successful OLT): the SHIVC, PV, IHIVC, HA, and BD. **Table 1 and 2** summarize the reconstruction methods for the hepatic venous system (SHIVC, PV, and IHIVC), the BD, and HA[20–22,24–48]. Generally, 5 methods are used for the reconstruction between the donor's and recipient's vein, artery, and BD: 1) microsuture, 2)

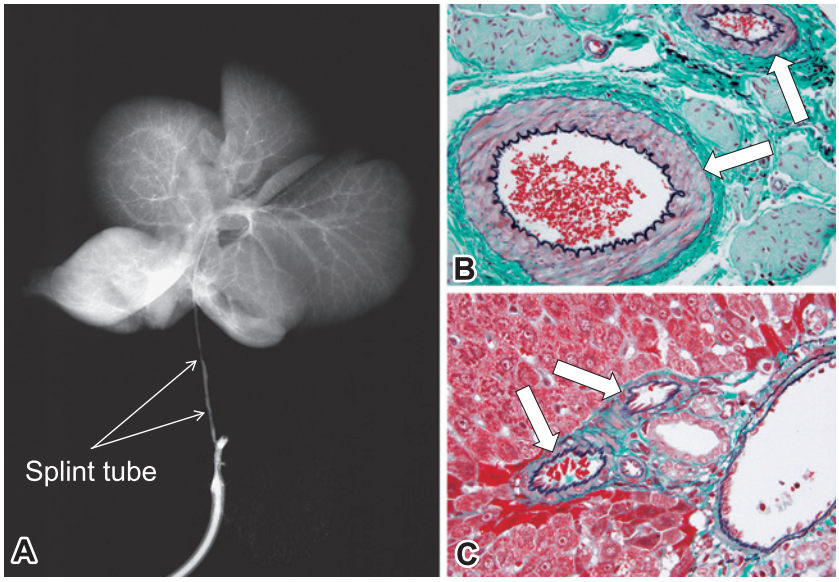

Fig. 6 Reconstructed hepatic artery in OLT.
A) Angiogram of the grafted liver through the recipient aorta showed good blood flow through the reconstructed hepatic artery (HA) 120 days after transplantation. Between **arrows** in Figure A, the splint tube was detected between the recipient right renal artery and donor celiac axis bearing the HA.
B, C) Histopathological findings of HAs in the hepatic hilus (**arrow** in B, ×400) and portal area (**arrow** in C, ×600) 360 days after liver transplantation with HA reconstruction showed that HAs were architecturally well preserved with red blood cells in their lumens indicating good hepatic circulation through the reconstructed HAs.

Table 1 Procedures of rat OLT without heparic artery reconstruction

Author	years	reference	PV	SHIVC	IHIVC	BD	HA
Lee	1975	21	suture	suture	suture	pull-through	non
Kamada	1979	22	cuff	suture	cuff	telescope	non
Zimmermann	1979	24	suture	suture	suture	splint	non
Miyata	1980	25	cuff	cuff	cuff	splint	non
Limmer	1981	26	cuff	suture	cuff	splint	non
Tsuchimoto	1987	27	cuff	cuff	cuff	?	non
Marni	1988	28	suture temporary splint	suture	suture temporary splint	splint	non
Xu	1992	29	cuff	suture	cuff	T-tube	non
Tan	2005	30	cuff	splint	cuff	splint	non
Oldani	2008	31	Quick-linker kit	Quick-linker kit	Quick-linker kit	telescope	non

PV, portal vein; SHIVC, suprahepatic inferior vena cava; IHIVC, infrahepatic inferior vena cava; BD, bile duct; HA, hepatic artery
The methods of suture, cuff, splint, telescope, sleeve, T-tube, and pull-through are indicated in **Fig. 6**.
?, uncertain of method of bile duct reconstruction
Quick-linker kit: The special microinstrument kit for the quick and easy connection of donor's and recipient's veins

cuff, 3) splint, 4) telescope, and 5) sleeve (**Fig. 7**). Especially, SHIVC, IHIVC, and PV are anastomosed mainly via a microsuture or cuff technique. With regard to the preferred surgical technique for rat OLT, most researchers have used 1 of the following 3 main models of hepatic venous system reconstruction; 1) microsuture of all veins (SHIVC, IHIVC, and PV), 2) the two-cuff (IHIVC and PV) method, and 3) the 3-cuff (SHIVC, IHIVC, and PV) method. The Kamada technique is a 2-cuff (IHIVC

Table 2 Procedures of rat OLT with hepatic artery reconstruction

Author	Year	Reference	PV	SHIVC	IHIVC	BD	HA	HA reconstruction	
								Donor	Recipient
Lee	1973	20	suture	suture	suture	pull-through	microsuture (E-S)	Aorta	Aorta
Engemann	1982	32	suture	suture	suture	cuff	microsuture (E-S)	Celiac a	Aorta
Lie	1984	33	suture	suture	suture	pull-through	microsuture (E-S)	Aorta	Aorta
Hasuike	1988	34	cuff	suture	cuff	splint	cuff	Aorta	Rt renal a
Howden	1989	35	cuff	suture	cuff	splint	microsuture (E-E)	Celiac a	Rt renal a
Steffen	1989	36	cuff	suture	cuff	splint	cuff	Celiac a	CHA
Chalaud	1990	37	cuff	suture	cuff	splint	telescope	Celiac a	HA
Liu	1992	38	cuff	suture	cuff	splint?	sleeve	Celiac a	Celiac a
Gao	1992	39	cuff	suture	cuff	splint	splint	PHA	PHA
Dippe	1992	40	suture	suture	suture	splint	microsuture (E-S)	Celiac a	Aorta
Zhao	1993	41	cuff	suture	cuff	splint	microsuture (E-S)	Aorta	Aorta
Sato	1996	42	cuff	suture	cuff	splint	sleeve	CHA	PHA
Li	2002	43	cuff	suture	cuff	splint	sleeve	CHA	PHA
Inoue	2003	44	suture	suture	suture	splint	microsuture (E-S)	Aorta	Aorta
Lehmann	2005	45	cuff	suture	cuff	cuff	splint	CHA	CHA
							cuff	CHA	Aorta
Ariyakhagorn	2009	46	suture	suture	suture	splint	splint	Aorta	CHA
Hori	2010	47	cuff	suture	cuff	splint	sleeve	CHA	PHA
							microsuture (E-E)	CHA	CHA
Huang	2011	48	cuff	suture	cuff	cuff	microsuture (E-S)	CHA	PHA
Ishii	2012	present	cuff	suture	cuff	telescope	splint	Celiac a	Rt renal a

PV, portal vein; SHIVC, suprahepatic inferior vena cava; IHIVC, infrahepatic inferior vena cava; BD, bile duct; HA, hepatic artery; Donor, donor artery using hepatic artery reconstruction; Recipient, recipient artery using hepatic artery reconstruction; microsuture (E-S), microsuture (end-to-side anastomosis); microsuture (E-E), microsuture (end-to-end anastomosis); The methods of suture, cuff, splint, telescope, sleeve, and pull-through are indicated in **Fig. 6**.
splint?, may be splint, but not described in detail
Celiac a, Celiac artery; Rt renal a, right renal artery; CHA, common hepatic artery; PHA, proper hepatic artery

and PV) method[22]. Although the microsuture technique provides conditions most similar to physiological conditions for the clinically transplanted liver and, thus, is associated with fewer complications, such as thrombosis[49], it requires a high level of microsurgical skill. In addition, the anhepatic time is longer in the microsuture technique than with the cuff procedure[26,31]. On the other hand, the complications reported with the cuff technique are blood flow disturbances, with subsequent thrombosis, and foreign body reaction to the cuff[36,49]. However, Kamada et al., who performed rat OLT 530 times over a 5-year period, reported a 95.3% survival rate of recipient rats[50]. In our experience, the long-term (>1 year) survival rate of the liver graft is 100% in Kamada technique-rat OLT for the syngeneic donor and recipient combination. Furthermore, the cuff technique, which reduces the anhepatic time (to less than 15 minutes) is favored for livers with a long preservation time[26,31,47]. A short duration of the anhepatic phase translates into successful OLT. The PV clamping time should not exceed 25 minutes, and IHIVC clamping time should not exceed 30 to 35 minutes. These time intervals are the thresholds for splanchnic and systemic venous cross clamping, beyond which cardiovascular depression and acid-base imbalances may ensue[28,49]. Several modified methods are also introduced for the reconstruction of SHIVC, IHIVC, and PV. To shorten the ischemic time, the temporary splint method is used for the anastomosis of the PV and IHIVC before the running suture technique (microsuture-temporary splint method)[28]. The splint method is also used to reconstruct the SHIVC[30]. In addition, for the quick and easy connection of hepatic veins (PV, IHIVC, and SHIVC), the special Quick-linker kit is used for their reconstruction[31]. The methods used for BD reconstruction include pull-through, telescopic,

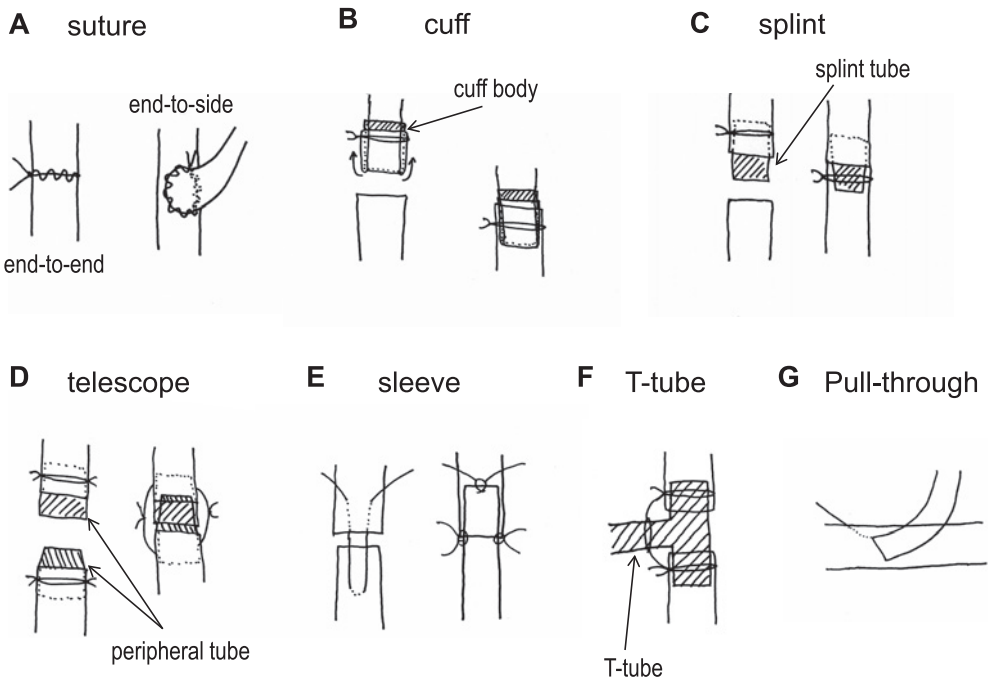

Fig. 7 The common methods for the anastomosis of vessels or bile duct in rat OLT.
A) The suture method includes the end-to-side or end-to-end running suture technique for the anastomosis of the donor and recipient veins and artery. B) Cuff method: A cuffed vein, artery, or bile duct is inserted into the corresponding vein, artery, or bile duct. C) Splint method: The splint tube is prepared in the donor vein, BD, or the recipient artery and connects the corresponding vein, bile duct, or artery. D) Telescope method: The peripheral tube is inserted in the donor and recipient bile duct or artery and connects their peripheral tubes to each other. E) Sleeve method: The recipient artery is inserted into the donor artery. F) T-tube method: For bile collection, the T-tube is inserted into the common BD, and the long arm is exteriorized from the abdomen. G) Pull-through method: The BD is tunneled into the lumen of the duodenum.

splint, and T-tube (**Table 1**, **Fig. 7**). For BD reconstruction, Teflon splints are commonly used, although the Kamada technique employs the telescopic technique for BD reconstruction[22]. To perform rat OLT more simply and easily and without complications, further modifications of surgical techniques will be needed in the future.

HA Reconstruction in Rat OLT

Kamada and coworkers[50] have considered arterial reconstruction in rat OLT to be unnecessary, and, therefore, the reconstruction is not part of the Kamada OLT technique. The need for re-arterialization varies greatly in different animal species. Reconstruction of the HA is considered an important step in liver transplantation in humans and large animals. Under normal conditions, the HA supplies at least 50% of the oxygen needed by the liver, which is essential for energy production and regulation of metabolism[51]. Accidental injury, occlusion, or both of the HA in liver transplantation in humans often results in graft loss, leading to re-transplantation, which is associated with high mortality rates[52–54]. In rats, the OLT recipient can tolerate the operation without arterialization, and the survival rate is high in these rats[50,55]. Therefore, the value of HA reconstruction in rat OLT has been debated[50,55]. However, previous studies have clearly demonstrated that arterialization in rat OLT increases survival[32,35,39], reduces microcirculatory disorders[56], avoids biliary complications[35,57] and histological changes[35,41,58], and alters immunologic responses[32,59]. Because HA reconstruction is routinely performed in clinical liver transplantation, we believe that HA reconstruction in rat OLT is necessary for the overall analysis of clinical transplantation.

Various techniques have been developed for hepatic re-arterialization in rat OLT (**Table 2**, **Fig. 7**)[20,32–48]. The publications described for rat OLT include the end-to-side microsuture anastomosis of the donor aorta, celiac artery, or common HA to the recipient aorta or proper HA; end-to-end microsuture anastomosis of the donor celiac artery or common HA to the recipient right renal artery after nephrectomy or common HA; cuff preparation in the recipient renal artery or the recipient common HA and connecting the donor aorta, common HA, or celiac artery; connecting the proper HA of the donor and the recipient or donor common HA and recipient aorta with a splint of polyethylene tubing; and microvascular sleeve anastomosis of the donor common HA and recipient proper HA or the celiac artery of the donor and the recipient.

As explained above, our splint technique from the recipient right renal artery to donor celiac axis bearing the HA is a simple and short procedure free of complications. With our method, arterial reconstruction in rat OLT is easy to perform and is not associated with prolongation of the entire procedure or with any harmful effect to the recipient. Thrombosis of the artery can be prevented (**Fig. 6**), and the reconnection procedure can be performed in seconds. Taking all aspects of rat OLT into account, we highly recommend the splint technique using the recipient right renal artery and the donor celiac axis bearing the HA to re-establish arterial perfusion of the graft.

Training and Learning Skills

The rat OLT model requires a skilled operator, and training for OLT is important for successful transplantation[60,61]. For complete training before experimentation, 40 to 50 OLTs per surgeon and more OLTs per nonsurgeon are necessary[47]. The Kamada technique for rat OLT is the standard method, and the splint technique of HA reconstruction is a simple procedure that is easy to learn and technically accessible for surgeons and nonsurgeons.

Conclusion

In this article, we described the techniques of Kamada for rat OLT and our recently developed splint technique for HA reconstruction. Both techniques are easy to perform and can be completed within a short period of time without major complications.

Although the microsuture technique offers the most physiologic method for anastomoses, a high level of microsurgical skill is a basic requirement. The most physiologic techniques are preferred for long-term survival studies, whereas the faster, simpler cuff techniques of rat OLT with HA reconstruction are useful for short-term as well as long-term survival studies. The procedure to be selected should be based on the defined study aims. We have used the rat OLT model to assess the pathology of liver graft rejection[62,63]. We anticipate a wider use of the rat OLT model and further research on complex issues related to liver transplantation, such as transplant immunology, and the mechanisms and treatment of inflammatory liver disease.

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