A half century (1961-2011) of applying microsurgery to experimental liver research

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INTRODUCTION

The development of microsurgery has been dependent on experimental animals. Microsurgery could be a very valuable technique to improve experimental models of liver diseases. Microdissection and microsutures are the two main microsurgical techniques that can be considered for classifying the experimental models developed for liver research in the rat. Partial portal vein ligation, extrahepatic cholestasis and hepatectomies are all models based on microdissection. On the other hand, in portacaval shunts, orthotopic liver transplantation and partial heterotopic liver transplantation, the microsuture techniques stand out. By reducing surgical complications, these microsurgical techniques allow for improving the resulting experimental models. If good experimental models for liver research are successfully developed, the results obtained from their study might be particularly useful in patients with liver disease. Therefore experimental liver microsurgery could be an invaluable way to translate laboratory data on liver research into new clinical diagnostic and therapeutic strategies.

Key words: Microsurgery; Portacaval shunts; Cholestasis; Hepatectomies; Liver transplantation; Portal hypertension

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Abstract

The development of microsurgery has been dependent on experimental animals. Microsurgery could be a very valuable technique to improve experimental models of liver diseases. Microdissection and microsutures are the two main microsurgical techniques that can be considered for classifying the experimental models developed for liver research in the rat. Partial portal vein ligation, extrahepatic cholestasis and hepatectomies are all models based on microdissection. On the other hand, in portacaval shunts, orthotopic liver transplantation and partial heterotopic liver transplantation, the microsuture techniques stand out. By reducing surgical complications, these microsurgical techniques allow for improving the resulting experimental models. If good experimental models for liver research are successfully developed, the results obtained from their study might be particularly useful in patients with liver disease. Therefore experimental liver microsurgery could be an invaluable way to translate laboratory data on liver research into new clinical diagnostic and therapeutic strategies.

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Microsurgery is a specific surgical field that requires the use of specialized microsurgical instruments, as well as dissection and suture microsurgical techniques. Microsurgical material is fundamental for performing microsurgical techniques. Microsurgical material is made up of means of magnification and microsurgical instruments[2].

The means of magnification or amplification systems are the basic and fundamental material for performing microsurgical techniques. In fact, the name of this kind of surgery is based on the use of magnification means. The amplification system par excellence in microsurgery is the surgical or operating microscope. The operating microscope is a binocular instrument with an optical lens system that provides stereoscopic or three-dimensional vision[2].

Microsurgical instruments have special characteristics for increasing the surgeon's ability in handling small structures while reducing tissue damage as much as possible. The micro instruments are designed to adapt to the surgeon's hand movements when using the operating microscope. The ergonomic characteristics or adaptation between hand and micro instruments are very important in microsurgery.[2].

Microsurgical instruments correspond to the fundamental time phases of a surgical operation: cut or incision, hemostasia, exposure, dissection and suture.

Hemostasia is carried out with hemostatic forceps and clamps. Clamps and vascular clips are instruments used for hemostasis and for bringing small vessels closer together without causing too much tissue damage[3]. New vascular clips have been designed to reduce trauma on the vascular wall[3]. Exposure to the surgical field is made up of separation, aspiration and traction. Forceps without teeth forprehension are the fundamental instrument for dissection. The fundamental instrument for suture is the needle holder.

Microsuture material is made up of microsutures and microsurgical atrumatic needles. Microsurgical materials can be reabsorbable in the long-term or non-absorbable, i.e. nylon or silk, and their gauge ranges between 6/0 (75 μ diameter) and 11/0 (14 μ diameter). Non-absorbable monofilament polypropylene and polyester synthetic suture material are also used[3,5].

In the beginning, surgeons should learn microsurgical techniques mentally and physically in order to perform them successfully. The maneuvers are usually slow and precise and last a long time. Therefore, the microsurgeon needs to be calm and rested when starting the procedure. During the operation, the setting should also be calm and relaxing. Distractions, such as noise and movements, should be avoided. To minimize fatigue, microsurgeons should work sitting down. The complete stability of the surgeon and the surgical field are essential to perform microsurgical techniques correctly[2].

### MICROSURGICAL TECHNIQUES FOR RESEARCHING LIVER DISEASES

Microsurgery applied to the rat and mouse liver makes it possible to obtain new experimental models and improve the already existing microsurgical models. From the pioneer works of Lee et al.[1,2,5] in the early 1960s through today, microsurgery has gained acceptance as an integral component of liver research.

Microdissection and microsutures are the two main surgical techniques that can be considered for classifying the experimental models developed. Based on this classification, simple and triple partial portal vein ligation, extrahepatic cholestasis and hepatectomies are all surgical models based on microdissection techniques, while in portacaval shunts and orthotopic and heterotrophic liver transplantation, the microsuture techniques stand out (Figure 1).

**Microsurgical dissection techniques**

The techniques of microsurgical dissection are mainly used for performing partial portal vein ligation, extrahepatic cholestasis and hepatectomies[8].

### PARTIAL PORTAL VEIN LIGATION

For the experimental study of portal hypertension (PH), the prehepatic type is usually chosen since it produces the least degree of hepatic insufficiency. The most frequently used experimental model of prehepatic PH is that achieved by simple partial portal vein ligation in the rat[9-11]. This surgical technique was first described by Chojkier and Groszmann in 1981[12]. In brief, after laparotomy, the portal vein is dissected and isolated. A 20 gauge blunt-tipped needle is placed alongside the portal vein and a ligature (4/0 silk) is tied around the needle and the vein. The needle is immediately removed, yielding a calibrated stenosis of the portal vein[10,12].

If it is taken into account that the intensity of the PH, as well as its posterior evolution, are conditioned by the resistance to the inflow produced by the constriction of the portal vein, this experimental model of prehepatic PH could be improved by increasing the initial resistance to the blood flow. With this objective in mind, we have modified the surgical technique by increasing the length of the stenosed portal tract with three equidistant partial ligatures. In brief, three partial ligatures are performed in the superior, medial and inferior portion of the portal vein and maintained in position by the previous fixation of the ligatures to a sylastic guide (Figure 2). The stenoses are calibrated by a simultaneous ligation (4/0 silk) around the portal vein and a 20 G needle. The midline abdominal incision is closed in two layers with an absorbable suture (polyglycolic acid) and 3/0 silk. The
EXTRAHEPATIC CHOLESTASIS

Obstructive jaundice causes a high rate of morbidity and mortality in the human clinical field\(^{13,15}\). The serious repercussions of cholestasis on the liver and at the systemic level have led to the creation of many experimental models in order to better understand its pathogenesis, prophylaxis and treatment.

Several surgical techniques for developing extrahepatic cholestasis have been described, especially in the rat, based on the section of the common bile duct between ligatures\(^{20,21}\). These macrosurgical techniques of extrahepatic cholestasis, called common bile duct ligation (BDL), cause the development of infected hilar biliary pseudocysts and multiple systemic abscesses and as a result, rats die during the early postoperative period due to sepsis\(^{18}\).

The hepatic parenchyma in the rat has four lobes, the right lateral, middle, left lateral and caudate lobes, which in turn have independent portal and arterial vascularization and a separate biliary drainage. This anatomic feature makes it possible to resect the bile ducts that drain the four lobes of the liver in continuity with the common bile duct up to the beginning of its intrapancreatic segment by means of a macrosurgical technique\(^{25}\) (Figure 3). An advantage of the macrosurgical technique of extrahepatic cholestasis in the rat is the absence of biliary pseudocyst formation, hepatopulmonary infection, and thus the prevention of mortality related to sepsis\(^{22,23}\).

In rats with microsurgical extrahepatic cholestasis, the weekly administration of antibiotics and vitamin K allows rodents to survive for more than 8 wk\(^{24,25}\).

In the long-term evolution, both macrosurgical (BDL) and microsurgical experimental cholestasis models develop hepatomegaly with a marked ductular proliferation and fibrosis, but the loss of normal liver architecture, typical of cirrhosis, is seldom found\(^{24,25}\). In relation to extrahepatic alterations, jaundice, choluria, PH with enlarged spleen and collateral portosystemic circulation, hepatic encephalopathy and ascites, stand out\(^{25,27}\). Therefore, experimental extrahepatic cholestasis is not only a good model for studying the hepatic pathology related...
HEPATECTOMIES

Hepatectomies in the rat allow for obtaining experimental models to study important aspects of hepatic physiopathology, such as liver regeneration or acute liver failure. Hepatectomies in the rat have benefited from the application of microsurgery because it reduces the limits to and the complications inherent in macrosurgical techniques. Knowledge of the rat's liver anatomy has been widened through the use of the operating microscope. In the classical descriptions of the rat liver, four lobes were normally considered: two big cranial or anterior lobes, the middle and the left lateral lobes, and two small caudal or posterior lobes, the right lateral and the caudate lobes. However, the findings obtained from the anatomical study using the operating microscope of the parenchyma located between the right lateral and caudate lobes allows for individualizing it as the caudate process (Figure 4).

The study of the distribution of the portal and vascular branches of the rat liver makes it possible to know its most frequent variations that must be considered when partial hepatectomies are performed. Furthermore, the distribution of these branches constitute the basis for the description of the functional anatomy of the rat liver as it defines the common site of the portal pedicles and the hepatic veins. Thus, anatomical-functional description is based on Couinaud’s description of the human liver. The unique aspects of rat liver anatomy allow for various degrees of resections and they are highly reproducible. Hepatectomies ranging from 5% to 95% of total liver weight can be easily performed with high reproducibility using microsurgical techniques because the parenchymal mass of each lobe is relatively constant. However, depending on the extent of the resection, the resulting experimental model has different degrees of usefulness. Therefore, depending on the rat liver lobe or segment that can be microsurgically resected, the most used techniques are those that allow for obtaining adequate models for studying liver regeneration and fulminant hepatic failure. Surgical removal of two-thirds (70%) of the liver in the white rat represents the most valuable and most extensively studied animal model of liver regeneration. In turn, subtotal (90%) hepatectomy represents an experimental model for studying acute liver failure.

MICROSURGICAL 70% HEPATECTOMY

Commonly, regeneration of the liver is studied by performing a surgical procedure that removes two-thirds of the liver mass in rodents (rats and mice), a technique known as two-thirds partial hepatectomy. This technique, which consists of the resection, after in “bloc” ligature, of the middle and left lateral lobes, was first described by Higgins and Anderson in 1931 to study the regeneration of the remaining parenchyma, the right lateral and caudate lobes.

Nowadays, this 70% hepatectomy technique can be improved with an operating microscope as the individualized dissection and ligation of the vascular and biliary branches of the middle and left lateral lobes can be done.
MICROSURGICAL 90% HEPATECTOMY

Subtotal (90%) hepatectomy invariably results in the death of rats if regeneration is not produced. However, this surgical model of hepatic failure does not reproduce the clinical setting of severe acute liver failure i.e. massive liver necrosis and cerebral edema. The 90% hepatectomy consists of the resection of the middle lobe, left lateral lobe and right lateral lobe. Short-term survival is possible when using a macrosurgical technique, with mass ligation or with a vessel-oriented approach. With this last technique and the subcutaneous administration of glucose immediately after the operation and the addition of glucose to drinking water to prevent hypoglycemia, a 1 wk 100% survival rate is possible.

This 90% hepatectomy technique can also be improved using an operating microscope. The microsurgical technique consists of the individualized dissection and ligation of the vascular and biliary branches from middle, left lateral and right lateral lobes without damaging the caudate process and caudate lobe branches (Figure 5).

In a 95% hepatectomy, the survival rate is one week (66% of the animals) and in a 97% hepatectomy it does not exceed 4 d. These techniques of subtotal or extended hepatectomies, when performed by microsurgery, reduce the injury to the caudate process parenchyma, These models of 90%, 95% and 97% liver resection are of great interest in studying the physiopathological mechanisms leading to the failure of the remnant liver, as well as in assaying new therapies that favor liver regeneration and subsequently increase the survival of the rat.

MICROSURGICAL TOTAL HEPATECTOMY

The total removal of the liver, while maintaining the portal and inferior vena cava circulation, provides a reproducible experimental model of acute liver failure. Total hepatectomy in the rat requires constructing a microsurgical portacaval shunt because this animal can only tolerate portal flow interruption for a maximum of 20 min.

Various techniques for creating an anhepatic rat have been described. Total hepatectomy in the rat can be performed in one, two or three stages. One-stage procedures consist of portacaval shunting and hepatectomy with prosthetic or vascular grafts. To avoid grafting, multistage procedures have been devised, where initially partial constriction of both the portal vein and the vena cava is carried out to establish an adequate collateral circulation. Microsurgical techniques can be used to obtain an anhepatic rat, not only to perform the portacaval shunt, but also to perform a total hepatectomy, avoiding damage to the intrahepatic vena cava.

MICROSURGICAL SUTURE TECHNIQUES

The techniques of microsurgical suturing are mainly used to make portacaval shunts, orthotopic liver transplants and heterotopic liver transplants.

Portacaval shunts

The splanchnic venous circulation flows into the portal vein towards the liver. The portal venin (‘porta’ means door in Latin) gives this venous system its name. The portal venous system creates a functional unit between the organs that drain and vascularize.

Microsurgery allows for carrying out different types...
of portosystemic shunts in the rat and mouse. The most frequently used have been end-to-side portacaval shunt, side-to-side portacaval shunt, mesentericocaval shunt and portacaval transposition.\cite{52,53}

End-to-side portacaval anastomosis in the rat is a shunt procedure that has become increasingly widespread since it was first performed by Lee et al.\cite{1,6} in 1961. Its value mainly lies in the fact that it is an appropriate technique for microsurgical training and for researching liver diseases, particularly hepatic encephalopathy.\cite{54,55,56}

Lee’s description of end-to-side portacaval shunt in the rat has served as a reference for authors to make modifications.\cite{9,13}. Thus, we have described a simplified end-to-side portacaval shunt in rats, which consequently favors its widespread use.\cite{32,33}. In brief, the portal vein is clamped at the confluence with the splenic vein and ligated (7/0 silk) at the level of its hilar bifurcation. Next, a partial venotomy is performed just below its hilar ligature. A 9/0 nylon thread with a loop at its distal end is used to perform the end-to-side shunt. The first stitch in the vascular suture is made before completing the portal vein venotomy. This stitch is passed through the upper angle of the hole in the vena cava. On completing the section of the portal vein, the loop brings the two vessels closer together and the shunt is continued without requiring a first knot to be made. On finishing the anastomoses by continuous running suture, the suture is tied when it reaches the end corner, the loop can be untied by pulling on the end and then the suture is tied to it\cite{52,53} (Figure 6).

Eck's fistula has been a model of liver atrophy for more than 100 years. The arterIALIZATION of the portal stumps after portacaval shunt in the rat prevents hepatic atrophy.\cite{96}. In this model, the metabolic, neurological and behavioral alterations could be similar to those found in human type B hepatic encephalopathy, which concerns encephalopathy related to the portosystemic shunt.\cite{55}. It has been proposed that the alterations produced in hepatic encephalopathy could be of an inflammatory nature.\cite{96}

To study these portacaval shunt experimental models, sham-operated rats are usually used as a control. The sham operation consists of portal vein clamping during a similar period of time to that of the portacaval shunt.\cite{96}

Orthotopic liver transplantation

The orthotopic liver transplant technique in the rat is very laborious, which is why it requires prior training. In the description by Lee et al.\cite{1,6}, they use the manual suture to perform the anastomosis, although the application of the cuff technique by Kamada and Calne in 1979\cite{99} has been more widely accepted as it simplifies the orthotopic liver transplantation\cite{50,51}.

**DONOR OPERATION**

The dissection of the infrahepatic inferior vena cava (IH-IVC) includes the sectioning between ligatures of the right lumbar adrenal veins, as well as the right renal vein. To dissect the entire length of the portal vein, the gastroduodenal vein is ligated with 10/0 nylon suture and sectioned. Hypothermic perfusion of the liver is carried out through the portal vein. The operation is completed with a sectioning of the common bile duct hepatic artery, the portal vein, the IH-IVC and the suprahepatic-inferior vena cava (SH-IVC), which is carried out at the intrathoracic level, respecting a diaphragmatic flap around it\cite{1,9,13}.

**Figure 6 End-to-side portacaval shunt in the rat.** The construction of a loop at the end of the suture facilitates the early approximation between the portal vein and the inferior vena cava, as well as its ending. Thus, when it reaches the end corner, the loop is untied by pulling on the end and the suture is tied to it. PV: Portal vein; IVC: Infrahepatic inferior vena cava.

In vitro preparation or “bench” surgery

Once the donor liver exeresis is performed, it is placed in a container with a hypothermic solution. Firstly, the common bile duct is perfused with 2 or 3 mL of cold preservation solution. Subsequently, a cuff is placed on the IH-IVC and then tied with a circumferential 6/0 silk ligature. Also, a cuff is tied on the portal vein with a circumferential 6/0 silk ligature.

**RECIPIENT OPERATION**

The recipient operation consists of a hepatectomy and transplant. A laparotomy is performed and the gastrointestinal tract is pushed downward towards the left of the animal. The hepatic artery is dissected and ligated, while the common bile duct is cannulated distally with a catheter and tied with a circumferential 6/0 silk ligature. Next, the portal vein is clamped and its right and left branches are ligated with 6/0 silk, sectioning the ligatures distally. The SH-IVC is clamped with a small Satinsky clamp. The caudal traction of the liver facilitates this maneuver. The sectioning of the SH-IVC at the level of the hepatic parenchyma finalizes the hepatectomy in the recipient.
The transplant beginning with an end-to-end anastomosis of the SH-IVC is performed by manual suture with Prolene® 6/0 thread. Later, the portal end-to-end anastomosis is performed using the cuff technique. Once the anastomosis has been ended, the portal vein and the SH-IVC are unclamped, while the liver progressively begins to regain normal coloring. The end-to-end anastomosis of the IH-IVC is also performed using the cuff technique. Next, the recipient IH-IVC is unclamped. Lastly, the cannula of the donor common bile duct is introduced into the lumen of the recipient common bile duct, thus performing an end-to-end cholecystocholedochostomy, over which the greater omentum is placed to avoid fistulas.

The arterial blood supply of the transplant could be restored through an aortic segment of the donor in continuity with the celiac axis and the hepatic artery. This donor aortic segment is sewn end-to-side with 10/0 monofilament thread to the recipient infrarenal aorta (Figure 7).

A mechanical microvascular anastomosis for orthotopic liver transplant in the rat using a quick-linker technique has been also proposed.

One of the most serious complications after orthotopic liver transplantation is primary non-function from ischemia-reperfusion injury. Hepatic microcirculatory disturbance secondary to inflammation after graft reperfusion produces hepatocyte damage.

Microsurgical models of orthotopic liver transplantation in the rat are an indispensable component of transplantation research. These experimental models serve to study new preservation methods, tolerance induction, rejection mechanisms and novel immunosuppressor therapies. The use of these microsurgical methods in liver transplantation avoids complications related to the surgical technique. When the surgical complications are minimized, complications associated with preservation, tolerance induction, rejection and new immunosuppressive drugs can be better studied.

**HETEROPTIC LIVER TRANSPLANTATION**

Heterotopic liver transplantation (HLT) is an alternative to orthotopic liver transplant in patients. The liver transplant is not performed in the same anatomical place. In most cases, the recipient liver is respected and, if so, the transplanted heterotopic liver is called the “auxiliary liver.” The HLT is a valid alternative to orthotopic liver transplant in both acute hepatic insufficiency and chronic hepatic insufficiency. However, better knowledge is still necessary in terms of postoperative complications to facilitate its diffusion to the human clinical area.

The development of microsurgical techniques was the decisive factor for disseminating the realization of the HLT in rats. In this animal, several techniques have been described for HLT that are differentiated by the vascularization of the graft (portal, arterial or arterio-portal), the venous drainage (through the SH-IVC or the IH-IVC), the type of portal blood (splanchnic or systemic), the biliary drainage (by choledochocholedochostomy or choledochojejunostomy), the localization of the graft (intra- or extra-abdominal) and the mass of hepatic pa-
renchyma (total liver or partial liver transplant).

PARTIAL HETEROOTOPIC LIVER TRANSPLANTATION

The donor operation is similar to that described previously in orthotopic liver transplant. But, in the hepatic hilum, the vascular structures and the bile ducts corresponding to the middle and the left lateral lobes are ligated when a partial heterotopic liver transplant is performed. In this case, the interlobular IVC, between the cranial and caudal lobes, is also ligated. The abdominal aorta is clamped at its proximal end and is cannulated at its distal end. Then, hepatic arterial perfusion is initiated with a hypothermic preservation solution. The effluent drains through a venotomy carried out in the IH-IVC. Next, the donor liver perfusion is initiated through the portal vein. Lastly, the graft is explanted. The in vitro preparation, or “bench surgery”, of the graft consists of placing a cuff on the portal vein and a ligature or suture on the proximal end of the abdominal aorta.

In the recipient, the portal vein is dissected along its whole length and the gastroduodenal vein is ligated and sectioned. The IH-IVC is dissected between the drainage of the renal veins. The ends of the dissected IH-IVC are clamped. On the anterior wall of the clamped IH-IVC, a 4mm long oval venotomy is carried out. The graft is removed from the hypothermic container and placed in the abdominal cavity of the recipient with the right lateral lobe cranially and the caudate lobe caudally. An end-to-side anastomosis is carried out by microsurgical suture technique between the IH-IVC of the donor and the IH-IVC of the recipient using 8/0 monofilament thread.

Once the anastomosis is completed, a bull-dog is placed on the donor IH-IVC to avoid reflux of venous blood into the graft when the recipient IH-IVC is unclamped to avoid hypovolemic shock. Next, the end-to-end portal anastomosis is carried out using the cuff technique. Once the graft is revascularized, a continuous running suture (bottom). p: Portal vein anastomosis; a: Aortic anastomosis; v: Inferior vena cava anastomosis. IVC: Inferior vena cava anastomosis; PV: Portal vein.

CONCLUSION

It could be concluded that the microsurgical techniques, when applied to experimental liver surgery, improve the experimental models obtained since they reduce the complications inherent in macrosurgical techniques. If good experimental models of liver research are successfully developed, the results obtained from their study might be particularly useful in patients with acute and chronic liver diseases.

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