Assessment and preservation of liver function in hepatic ischemia and reperfusion
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Citation for published version (APA):
Heijnen, B. H. M. (2003). Assessment and preservation of liver function in hepatic ischemia and reperfusion
Chapter 3

Experimental liver resection.

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In Liver Surgery: Operative techniques and avoidance of complication
**Introduction**

When reading dr. Pringle’s landmark publication in the Annals of Surgery of 1908 in which he relates the successful occlusion of the afferent vessels to control severe liver bleeding, it becomes apparent that it was only with great concerns that he attempted this manoeuvre (1). He thought that clamping of the vessels to the liver was a highly risky undertaking because animal experiments reported in the German literature at that time, had shown disastrous results from obstructing the hepatic and portal vessels leading to immediate collapse of the animal or death in short time. These animal experiments might have been well devised but the conclusions drawn from these studies were wrong, because we know now that these animals did not die of liver ischemia but succumbed because of intestinal congestion secondary to occlusion of the portal vein. Fortunately, in humans collateral circulation of the portal venous system is better developed and allows occlusion of the hepatic pedicle for considerable time. Hence, one should be cautious when drawing conclusions for the clinical, human setting on the basis of animal experiments. Nevertheless, the current techniques in liver resection have in part been derived from animal experiments which have improved our understanding of how to handle the liver during major liver surgery. This section deals with experimental liver resection, paying special attention to models in several animal species, techniques of temporary vascular inflow occlusion and total vascular isolation of the liver, and to some of the answers these studies have provided in relation with the sequelae of ischemia and reperfusion of the liver.

**Comparative anatomy of animal livers**

Experimental liver resections can be performed in either small or large animals. Clearly, the use of small animals has important advantages in terms of availability, costs, laboratory management, anesthesia and the possibility of using well defined strains of biologically identical animals. Because of the similarity in histoarchitecture of rat and human liver, the rat liver has been extensively studied in various models relating to liver physiology (2). The choice of large animals has shifted more and more from the use of dogs to the use of pigs for obvious reasons as costs and public opinion. For liver resection studies encompassing vascular occlusion procedures, dogs unlike pigs and humans, have the additional disadvantage of well-developed sphincters in the hepatic veins that constrict in response to ischemia and may give rise to the phenomenon of outflow block (3). Resection models in large animals are clinically more relevant in view of the
volume and weight of the liver, similarity of surgical procedures and impact on hemodynamic parameters.

In the pig, the shape and position of the liver as well as its vascularization and histologic appearance are quite the same as it is in the human (4). There are again four major liver lobes, the left lateral and median lobes and the right median and lateral lobes. The caudate process extends medially of the right lateral lobe. The course of the extrahepatic biliary tract in pigs is well defined in the hepatoduodenal ligament, with the confluence of the left and right hepatic duct situated more distally than is usually the case in humans. The anatomy of the hepatic artery is quite consistent coming off from the celiac trunk which runs adjacent to the medial border of the portal vein. After giving off branches to the pancreas and stomach, the artery divides into branches to the right and left liver lobes in the porta hepatitis. The portal vein bifurcates or trifurcates in the porta hepatis and ramifies into the right and left liver lobes. Different from the human situation is the hepatic venous drainage into the vena cava which occurs through three large and several small veins directly into the vena cava. The intraparenchymal location of the hepatic veins hampers separate isolation of these veins during partial liver resection. The infrahepatic vena cava lies remarkably loose and is surrounded by a projection of liver parenchyma before taking its retroperitoneal position.

The dog liver is grossly divided into four lobes (5): The left lobe forms a third to one half of the total liver mass. The quadrate lobe lies in the median plan adjacent to the gall bladder fossa on its right side. The right lobe is much smaller than the left lobe and caudally overlaps the caudate process of the caudate lobe. The caudate lobe is composed of the caudate and papillary processes connected by a bridge of liver tissue. The portal bifurcation is similar to the situation in humans. Three or more hepatic veins drain into the suprahepatic vena cava and can be isolated extrahepatically before running into the vena cava.

As in most rodents, the rat liver is composed of four distinct lobes joined dorsally. The median lobe is most prominent and is divided by a cleft into a right and left portion. The median lobe is flanked by the right lateral lobe and the larger left lateral lobe. The caudate lobe is the smallest portion of the liver lying both dorsal and ventral to the esophagus (Figure 1). The common bile duct is formed by fusion of the hepatic ducts and lacking a gallbladder, has no capacity for the storage of bile (2).
The liver of the mouse is quite similar in its anatomy to the rat liver. Mice do have a gallbladder located in the confluence of the median lobe. The availability of genetically modified mouse strains have made these animals particularly interesting for the study of e.g., the inflammatory responses to partial liver resection.

Liver resection in pigs

Because the left portal vein is readily accessible in the porta hepatis, a left hemihepatectomy is usually preferred as standard partial liver resection in the pig (6). Through a midline laparotomy the hepatic pedicle is isolated and if required, the common bile duct cannulated to assess postoperative bile production. All peritoneal connections of the liver to diafragm and posterior abdominal wall are divided. The left branch(es) of the hepatic artery and portal vein are ligated and divided resulting in discoloration of the left liver lobes, thereby defining the resection plane. The line of resection runs inbetween the left and right medial lobes, just left of the gallbladder fossa. The left branch of the extrahepatic bile duct is ligated near its confluence. To limit blood loss, parenchymal dissection is best performed under total vascular exclusion: After clamping of the hepatic pedicle (portal vein and hepatic artery) and the suprahepatic and infrahepatic caval vein, a left hemihepatectomy is performed using an electrosurgical knife. Hemostasis at the resection margin is reliably achieved by application of 5 millilitres of fibrin glue (Tissucol®, Baxter, The Netherlands) at the raw surface, followed by suture closure of the resection plane.
After completion of the resection, circulation in the suprahepatic and infrahepatic caval vein is restored and the hepatic pedicle clamp released. The average time of this resection technique is 10 minutes and should not be extended lest hypotension and acidosis occur due to splanchnic venous congestion secondary to prolonged occlusion of the portal vein. The mean percentage of liver tissue resected by standard left hemihepatectomy in the pig is approximately 45%.

Assessment of liver function and damage after experimental liver resection

Parenchymal damage of the liver is classically assessed by determination of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) in the blood. Glutathion-s-transferase (GST) has proven a more sensitive parameter for hepatocellular injury during ischemia than AST or ALT. An additional advantage of GST is that only small amounts of plasma (5μl) are required for its determination which enables repeated assessments in rats and mice (7). Bile production is a parameter of excretory liver function and can be monitored continuously as final proof of liver function. Microvascular injury can be assessed by measurement of intrahepatic tissue pO₂ and determination of sinusoidal endothelial cell damage. Intrahepatic tissue pO₂ measurements using the polarographic pO₂ needle electrode method as described previously, has the advantage of yielding a pO₂ histogram constructed from 100 consecutive pO₂ values obtained by stepwise withdrawal of the needle over a 2 cm tract in the liver parenchyma (8,9). Mean values of intrahepatic tissue pO₂ as well as pO₂ distribution patterns (CFDC) can be computed. A method for evaluation of SEC damage devised in our laboratory, consists of the ability of the SECs to take up exogenous hyaluronic acid after reperfusion, as compared to baseline uptake capacity of hyaluronic acid before ischemia (10,11). Bile production is monitored as a parameter of excretory liver function. As a metabolic liver function test, the galactose elimination capacity of the liver before and after liver resection can be determined. Alternatively, indocyanine green elimination can be assessed which test is more a function of microvascular tissue perfusion (6,12,13).

Vascular inflow occlusion methods in the pig

Since massive blood loss and function of the remnant liver are major concerns when performing extensive liver resections, a number of experimental studies have focussed on temporary vascular inflow occlusion methods to reduce blood loss. Arrest of circulation in the liver translates into hepatocellular ischemia and subsequent reflow into parenchymal reperfusion. The sequelae of
vascular inflow occlusion in combination with liver resection can therefore be studied as post-ischemic, reperfusion phenomena. The pig liver is remarkably tolerant to normothermic ischemia, a finding reported previously by Nordlinger who found no significant increase in hepatic enzyme release in pig livers after 90 minutes of ischemia and subsequent reperfusion (3). In our laboratory, 120 mins of ischemia in which time a left hemihepatectomy was performed, resulted in substantial hepatocellular and sinusoidal endothelial cell damage. As in most animals, however, splanchnic congestion due to portal vein occlusion is not tolerated and necessitates the construction of a portal-caval shunt during prolonged hepatic pedicle clamping. A portal–caval shunt can be created by interposition of a 10 mm Dacron graft between portal and caval vein, which can be clamped or left open according to the portal vein being open or occluded (Figure 2).

Using this model, the hypothesis was tested whether intermittent clamping of the hepatic pedicle during prolonged vascular inflow occlusion was less injurious to the liver than continuous clamping. The outcome was that continuous clamping resulted in less microcirculatory and hepatocellular damage compared to intermittent clamping, when vascular inflow occlusion was applied for 90 minutes. However, when vascular inflow occlusion was extended to 120 minutes, intermittent clamping of the hepatic pedicle was the preferred method resulting in less injury during liver resection (14).

Favorable results have been claimed of total vascular occlusion in combination with cooling of the liver during extended liver resection procedures (15-17). Hypothermic protection of the liver during ischemia is a well known strategy in the transplantation setting, when donor livers are flushed with an appropriate organ preservation solution and stored at 4°C. Experiments in which the liver is cooled in situ for resection, require not only total vascular isolation of the liver,
but also free drainage of the cooling solution from the hepatic veins. If the cooling solution is allowed to enter the circulation, not only will a systemic drop of temperature occur, but also the components of the flush solution, with usually a high potassium content, constitute a potential hazard. In our laboratory, experiments are conducted in which the liver is cooled in situ under complete vascular isolation. A device is used consisting of a two-way, perspex prosthesis which is introduced through a venotomy of the infrahepatic vena cava and advanced into the caval vein above the level of the diafragm. Ties around the suprahepatic vena cava between liver and diafragm at the cranial end of the prosthesis, and around the infrahepatic vena cava (above the renal veins) at the caudal end, secure the prosthesis. The lumen of the prosthesis provides continuous venous drainage of the lower caval system while excluding the hepatic veins and at the same time receives the portal venous blood from a shunt inserted in the portal vein and connected to a side-port of the prosthesis (Figure 3).

**Figure 3**
Experiments under complete vascular isolation of the pig liver are conducted by using a portal-caval, intraluminal prosthesis. This device consists of a two-way, perspex prosthesis which is introduced through a venotomy of the infrahepatic vena cava and secured at the level of the diafragm. The prosthesis provides continuous venous drainage of the lower caval system while excluding the hepatic veins. Portal venous blood is drained via a shunt connected to a side-port of the prosthesis. Prior to left hemihepatectomy, the right hemi-liver is flushed by retrograde canulation of the divided, left hepatic artery.

The flush solution after passing through the hepatic veins, leaves the caval vein via the venotomy. Circulation is reestablished by releasing the tie around the suprahepatic vena cava and placing a tie around the infrahepatic vena cava cranial to the venotomy, or removal of the prosthesis and closure of the venotomy.

**Liver resection in dogs**
Partial liver resections in dogs are carried out more or less along the same lines as in pigs, and in humans. Usually, a left hemihepatectomy is performed resulting in resection of around two-thirds of the whole liver volume. After division of the left hepatic artery and left portal vein the line of demarcation will appear just right to the quadrate lobe and the gallbladder fossa, and left to the
right medial lobe. A bridge of parenchyma, most of which consists of the body of the caudate lobe between its caudate and papillary processes, connects the left and right liver lobes and is dissected for separation of both sides of the liver. The hepatic veins, usually a left and right hepatic vein with a smaller vein adjacent to the left hepatic vein confluence, are readily dissected before entering the suprahepatic vena cava.

Liver resection and induction of liver ischemia in the rat

A midline laparotomy is performed extending from below the xiphoid cartilage. The transparent, falciforme ligament between the convex face of the liver and the diaphragm is cut down to the suprahepatic vena cava. For liver resection, the median and left lateral lobes are mobilized and pushed out of the abdominal cavity. Two suspensory ligaments, one attaching the dorsal face of the left lateral lobe to the median blood vessels and to the stomach, and the other, attaching the anterolateral edge to the posterior peritoneum, are divided. A ligature is tied around the base of the blood vessels to the median and left lateral lobes after which both lobes are cut near to the ligature. Within 10-20 days post-resection, the liver will be found to have fully regenerated (18).

Liver ischemia can be induced partially or in the whole liver, followed by reperfusion. Total liver ischemia as results from clamping of the hepatoduodenal ligament will lead to splanchnic congestion, which is badly tolerated in rodents. Partial liver ischemia by clamping of the portal and arterial branches to the median and left lateral lobes is better tolerated because portal outflow via the right lateral lobe remains secured. After restoration of vascular inflow, the right lateral lobe may be excised leaving only the reperfused liver portions for assessment of post-ischemic damage (19,20).

Prolonged ischemia in the rat requires appropriate anesthesia and monitoring of vital parameters and arterial pO₂ to prevent tissue acidosis which influences ischemic tolerance of the liver. Preferably, tracheal intubation and continuous ventilation with volatile anesthetics are applied while continuously monitoring end-tidal CO₂, to maintain physiological pH during the entire procedure (21). In a rat liver resection model of limited (30 minutes) ischemia, improved results following continuous vascular inflow occlusion were found as compared to intermittent clamping (19). However, in a cirrhotic liver model or when applying prolonged periods of ischemia, a benefit from intermittent hepatic vascular inflow occlusion was found although in the latter experiments, no partial liver resection was performed (22-24).
Conclusion

Models of liver resection, in small animals (rats, mice) as well as in large animals (pigs, dogs) have proven valuable in increasing our understanding of the mechanisms of the local and systemic injurious effects associated with major liver surgery. Especially the sequelae of liver ischemia and reflow resulting from temporary vascular inflow occlusion, with or without caval vein isolation, have been the subject of several experimental studies. The major limitation of animal studies concerning liver resections are, apart from a species difference in ischemic tolerance of the liver, the inability of the venous splanchnic system to sustain portal vein occlusion. Unlike in humans, in which the naturally occurring portal-systemic collateral circulation allows prolonged clamping of the portal vein, animals require construction of a portal-caval shunt to decompress the splanchnic venous system during occlusion of the portal vein for longer than a few minutes. Similar precautions need to be observed in experimental liver resections as in human liver surgery, i.e. prevention of major blood loss and postoperative insufficiency of the remnant liver. Application of fibrin glue (Tissucol®, Baxter) on the liver resection surface in large animals has proven extremely helpful in reducing intraoperative and postoperative bleeding complications. When performed under standardized conditions and assessed according to validated criteria, experimental liver resections can contribute substantially to improvement of techniques and safety in clinical liver surgery.
References


