Hemodynamic tolerance and rapid hypertrophy of a hepatic graft corresponding to less than 30% of the ideal mass in pigs

Michel POUYET (1), Christian PAQUET (2), Isabelle MECHET (3), Yannick LE DERF (3), Pierre BERNARD (3), Paolo FIGUEIREDO (3), Françoise BERGER (4), Olivier BOILLOT (3)

(1) Faculté Laennec, Lyon ; (2) Laboratoire de Physiologie, École Vétérinaire de Lyon, Marcy-l’Etoile ; (3) Unité de Transplantation Hépatique, (4) Laboratoire d’Anatomie Pathologique, Hôpital Edouard-Herriot, Lyon.

SUMMARY

Background and objective — Evaluation of a new pig liver transplantation technique for survival and hypertrophy of a small-sized graft by providing adapted and controlled venous portal flow.

Material and methods — Twenty Large-White pigs underwent heterotopic liver transplantation after a mesocaval shunt and ligation of the superior mesenteric vein downstream from the shunt. The donor-to-recipient weight ratio was below 30%. Furthermore, recipient’s biliary duct and portal vein into the hilum were tied. In a control group, no mesocaval shunt was performed and the graft received the entire splanchnic venous flow.

Results — The mesocaval shunt provided diversion of 60% of the splanchnic blood flow. The median survival of study pigs was 39 days (range: 8-98). Median serum bilirubin levels at 1 week were 12 µmol/L (range: 4-59). At autopsy, graft weight was increased to 2.7 times the initial weight and histological findings were normal. In the control group, all pigs died quickly from acute splanchnic congestion.

Conclusion — In a model of heterotopic liver transplantation using small-sized grafts, complete diversion of mesenteric blood flow through a mesocaval shunt resulted in hemodynamic tolerance and hypertrophy of a graft corresponding to less than 30% of the ideal mass.

Key words: Heterotopic liver transplantation. Small-sized graft. Mesocaval shunt. Pigs.
so the grafts received the full splanchnic venous ligature of the native portal vein downstream from the portal anastomosis. The same technique was performed in 4 pigs at 1 month using the ONO protocol (1.5 mL/kg of 1% CINH₄ in 30 minutes, venous ammoniemia at 0, 30, 60, 120 and 180 minutes) [12].

Animal survival (spontaneous death or sacrifice), graft weight at autopsy, and histology of the transplanted and native livers were also recorded.

Results

Mean donor-to-recipient weight ratio was 0.24. Mean GRWR was 0.6. For the donors, the liver-to-body weight ratio was 2.4%. Transposing this ratio to the recipient animals weighing a mean of 30 kg, the standard graft-to-recipient weight ratio was 24%.

Peroperative venous flow

Mean portal flow into the native liver was 747 mL/min (range, 452–1,115). Flow to the graft was 304 mL/min (range, 151–452), and in the mesocaval shunt it was 443 mL/min (range, 241–663). The terminalized mesocaval anastomosis shunted 60% of the splanchnic venous flow to the inferior vena cava with the graft only receiving 40% of the entire splanchnic flow.

Function tests

Bilirubin, aminotransferase, alkaline phosphatase, and residual cyclosporin values are summarized in Table I. Serum bilirubin normalized at one week. The hyperammoniemia test performed one month after liver transplantation in 4 pigs showed a normal clearance curve (Table II).

Table I. – Results of biological parameters in study pigs (values in medium and range)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1st week (N &lt; 20)</th>
<th>3rd week (N &lt; 50)</th>
<th>5th week (N &lt; 200)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bilirubin (µmol/L)</td>
<td>12 (4-59)</td>
<td>10 (4-76)</td>
<td>9 (3-101)</td>
</tr>
<tr>
<td>ALAT (UI/L) (N &lt; 50)</td>
<td>24 (15-55)</td>
<td>28 (13-60)</td>
<td>31 (5-41)</td>
</tr>
<tr>
<td>ALP (UI/L) (N &lt; 200)</td>
<td>151 (66-318)</td>
<td>143 (68-410)</td>
<td>157 (74-349)</td>
</tr>
<tr>
<td>Residual cyclosporin (ng/mL)</td>
<td>113 (28-300)</td>
<td>113 (9-405)</td>
<td>114 (46-282)</td>
</tr>
</tbody>
</table>

ALAT: alanine aminotransferase; ALP: alkaline phosphatase; N: normal.

Table II. – Results of intravenous ammonia overload test in 4 pigs: progressive and physiological decrease in ammonia levels (µmol/L).

<table>
<thead>
<tr>
<th>Timing</th>
<th>Ammoniemia µmol/L (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TO = before perfusion</td>
<td>115 (100-142)</td>
</tr>
<tr>
<td>T1 = 30 min after end of perfusion</td>
<td>207 (179-231)</td>
</tr>
<tr>
<td>T2 = 60 min after end of perfusion</td>
<td>171 (144-179)</td>
</tr>
<tr>
<td>T3 = 120 min after end of perfusion</td>
<td>153 (133-164)</td>
</tr>
<tr>
<td>T4 = 180 min after end of perfusion</td>
<td>135 (123-147)</td>
</tr>
</tbody>
</table>

Ammoniemia: Normal < 100 µmol/L.

Operative techniques in the control group

Arteries, complete mobilization of the liver, cannulation of the superior mesenteric vein, then aortic clamping followed by liver cooling with 1 liter UW solution via the portal vein. The graft was then removed with the arterial trunk to the celiac aorta and weighed. The intra-pericardic vena cava, mesocaval anastomosis with a venous graft (donor inferior vena cava, mesocaval anastomosis in the recipient.

After harvesting, the recipient was opened by supra- and infra-umbilical mid-line incisions. The transplantation was done with a mesocaval anastomosis terminalized by ligature of the superior mesenteric vein downstream; likewise the native liver portal vein was ligatured close to the hilum so the graft only received venous flow from the gastro-duodenosplenopancreatic circuit (Figure 1). Truncular vagotomy with gastroenterostomy was performed to prevent ulcerative complications and the native common bile duct was ligatured.

Briefly, the procedure was as follows: truncular vagotomy, section between the ligatures on the native common bile duct, dissection of the portal vein, the superior mesenteric vein, and the subhepatic inferior vena cava, mesocaval anastomosis with a venous graft (donor inferior vena cava) during temporary clamping of the superior mesenteric artery, installation of the heterotopic graft under the native liver with end-to-side anastomosis between the recipient subrenal aorta and the donor celiac aorta, then graft revascularization before installing the end-to-side porto-portal anastomosis. Mesenteric flow was then shunted by ligating the superior mesenteric vein downstream. The native portal vein was ligatured downstream from its anastomosis with the graft. In this way, the native liver was supplied with portal flow alone. The bile ducts were sutured to establish continuity between the graft gallbladder and the duodenum.

Study parameters

Peroperative venous flow was measured with Doppler (Doppler Basic, ATYS Medical, Le Frontagny, Saint-Genis-Laval, France) in 10 pigs in order to assess portal flow reaching the native liver both in the normal condition and after venous ligatures, shunted mesocaval flow, and flow reaching the graft.

Abreviations: GRWR: graft-to-recipient weight ratio.
Follow-up

Median survival in the 20 study animals was 39 days (range, 8-8). Twelve animals survived longer and were sacrificed at 52.5 days (range, 30-98) after transplantation.

Eight pigs died spontaneously with a median survival of 18 days (range, 8-4), 5 due to sepsis and 3 due to digestive complications. Survival in the control group was less than 12 hours for 2 animals and 1 week in 3 animals. All animals in the control group died from acute portal hypertension.

Autopsy data

The vascular anastomoses were patent in all pigs. There were 3 biliary fistulae, 2 intestinal occlusions, and 1 perforation of a gastric ulcer. At autopsy, mean weight of all the pigs and those that survived less and more than 30 days were 28, 24 and 33 kg respectively. Graft weight at autopsy is given in table III. Hepatic hypertrophy reached a mean 2.7-fold increase from the initial weight: 1.8-fold in pigs that survived less than 30 days and 3.36-fold in the 12 pigs that survived more than 30 days. Likewise, GRWR rose from 0.6 initially to 1.7 at autopsy for all studied pigs.

Histology findings

A histology examination was performed on all native livers from pigs surviving more than 30 days. There was minimal lobular infiltration by polymorphonuclear cells in 2 cases, and minimal centrilobular sinusoidal dilatation in 2 others. Moderate and minimal cellular rejection was observed in 5 and 1 cases, respectively. The histology examination was normal in the other cases, exhibiting a regenerative aspect and normal lobular architecture (figure 2). In the grafts implanted in the control pigs, there was evidence of massive congestion and acute lesions with hemorrhagic infiltration of the lobules and the portal spaces in 4 cases (figure 3). In one animal that survived 7 days, there was centrilobular necrosis, sinusoidal dilatation and cholestasis associated with an aspect of hepatocellular regeneration around the portal spaces. The native livers exhibited secondary biliary cirrhosis in 5 cases (figure 4). Portal and peri-portal fibrosis was observed in all cases with accentuated lobulation, enlargement of the portal spaces and polymorphonuclear cell infiltration and neoductal proliferation as well as signs of acute angiocholitis.

Discussion

Survival of a small-sized liver graft is closely related to rate of portal flow and thus portal pressure. If the transplanted liver mass is less than 50% of the ideal mass, it would be important, according to the results of this study and those reported by Ku et al. [6] to reduce portal flow. In the dog, approximately 60% of the portal flow comes from the mesenteric circulation [12]. According to our measures this percentage is 60% in the pig. It could exceed 75% in fasting humans [13], but would be around 50% in case of cirrhosis with portal hypertension [14,15]. The terminalized mesocaval shunt thus reduced portal flow by at least 50%.

Contrary to the surgical model reported by Ku et al. [6] in dogs implanted with a graft having one-quarter of the ideal mass using a porto-suprahepatic shunt where flow could not be controlled, our experimental model used a shunt perfectly controlling 60% of the splanchnic venous flow. Controlled venous flow into the graft is a crucial feature. When a small-sized graft

---

Table III. Initial graft weight and at autopsy.

<table>
<thead>
<tr>
<th></th>
<th>All pigs (n = 20)</th>
<th>Survivors &lt; 30 days (n = 8)</th>
<th>Survivors &gt; 30 days (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight (g)</td>
<td>180</td>
<td>182</td>
<td>175</td>
</tr>
<tr>
<td>Weight at autopsy (g)</td>
<td>489</td>
<td>343</td>
<td>594</td>
</tr>
<tr>
<td>Ratio (regeneration)</td>
<td>2.7</td>
<td>1.8</td>
<td>3.36</td>
</tr>
<tr>
<td>GWRW at autopsy</td>
<td>1.7 %</td>
<td>1.4 %</td>
<td>1.8 %</td>
</tr>
</tbody>
</table>

GWRW: graft weight recipient weight ratio.

---

Fig. 2 – Normal histological hepatic pattern in a study pig.

Fig. 3 – Liver graft in a control pig without mesenterico-caval shunt: acute massive congestion and haemorrhagic infiltration of lobules and portal tracts.

Fig. 4 – Native liver in a pig with long-term survival: secondary biliary cirrhosis.
receives all the splanchnic venous flow (the control animals here), the massive inflow produces irreversible lesions to the hepatic parenchyma comparable to those observed by Mann et al. [10] in rats. These authors found severe congestion and hemorrhagic infiltration, or to a lesser degree, centrilobular necrosis, and sinusoidal dilatation leading to irreversible cholestasis. By shunting the mesenteric venous flow via a terminalized mesocaval anastomosis controlling venous inflow, the graft receives the gastroduodenosplenic pancreatic venous flow more adapted to its mass.

The combination of a terminalized mesocaval shunt and implantation of a small-sized graft corresponding to 24% of the ideal mass produces a hemodynamic situation similar to that observed for a conventional transplantation with a graft at least 50% of the ideal mass but receiving the entire splanchnic venous flow, i.e. a perfectly tolerable hemodynamic situation [1-3].

Portal blood flow from the gastroduodenosplenic pancreatic circuit containing specific hepatotropic qualities described by Pouyet et al. [16], Starzl et al. [17] demonstrated the trophic effect of insulin. Moreover, hepatic mitogens arise at this level and play an essential role: HGF (hepatic growth factor) is essentially produced by the exocrine pancreas, EGF (epidermal growth factor) is also secreted by the pancreas and the Bruner glands in the duodenum [18]. This qualitative effect has been demonstrated in the dog where part of the liver received duodenopancreatic flow selectively and exhibited an absence of atrophy despite a 30 to 80% reduction in total flow [16]. The effect of this hepatotrophic pancreatic factor contained in gastroduodenosplenic pancreatic venous blood was also noted after allo- [19] and iso-transplantation [20, 21]. Yu et al. [22] however have suggested that part of these hepatotrophic factors could be synthesized in the small bowel mucosa.

The terminalized mesocaval shunt does not have any deleterious short-term or long-term effect on healthy liver tissue as demonstrated by Jolly et al. [23] in the dog and as observed by Reynolds et al. in one patient [24]. The results of the hyperammoniemia test in our series were normal compared with the results observed Jolly et al. [23]. In humans, a mesocaval shunt subsequent to portal atresia or thrombosis in children with a healthy liver and followed to adulthood have never developed general trophic or cerebral disorders due to portosystemic encephalopathy [25-29]. Warren et al. [30] demonstrated the persistence of a hepatopetal perfusion in case of portal cavernoma on a healthy liver because the intrasplenic pressure is greater than the intrahepatic sinusoidal pressure. The presence of a terminalized mesocaval shunt thus has no consequence when the graft is functional and is tolerated by effective immunosuppression. The experimental situation created here is however not exactly comparable with a simple portosystemic shunt because hepatopetal portal flow must theoretically be preserved after separating the gastroduodenosplenic pancreatic and the superior mesenteric sectors by ligature of the superior mesenteric vein.

It is quite noteworthy that in this model serum bilirubin (an expression of the graft’s clearance capacity) one week after transplantation was normal in all the animal with a favorable postoperative period. Despite their small size, the grafts were exposed to favorable hemodynamic conditions and had the opportunity to clear bilirubin produced by a body mass virtually four times larger than normal. In ordinary clinical situations, such a small-for-size graft would have invariably led to cholestasis with irreversible generalized jaundice [8].

Major and rapid graft regeneration-hypertrophy was observed. The GRWR tripled in slightly more than one month, nearly reaching a physiological ratio at autopsy. The hypertrophy observed was comparable to the regeneration observed after partial hepatectomy of a healthy liver in humans [31, 32]. In a model separating venous flow in a dog, liver hypertrophy of the lobe receiving the gastroduodenosplenic pancreatic flow was hindered because the other lobe receive the mesenteric flow and exhibited slow hypertrophy [16, 33]. Likewise, in a partial hepatectomy model with separated venous flow, Starzl et al. [33] found limited regeneration of the part of the liver receiving the gastroduodenosplenic pancreatic flow because of the persistent mesenteric supply to the contralateral lobe. In our study, the significant hypertrophy of the graft resulted from exclusive gastroduodenosplenic pancreatic venous supply and suppression of all portal supply to the native liver. The recognized mitogenic effect of cyclosporin was apparently accessory [34].

The high heterotopic assembly at the suprarenal level avoided supraphepatitic stasis as confirmed by the absence of signs at pathology examination. In addition, in quadruped animals, the pressure in the inferior vena cava is lower than in bipedal humans and the inspiratory depression is propagated as far as the renal veins [35]. Hess et al. [35] noted a minimal 0.2 mm H2O pressure differential between the suprarepatic hepatic and the suprarenal level of the inferior vena cava.

Conclusion

This study demonstrated the deleterious effect of excessive portal venous flow on small-sized grafts weighing less than 30% of the ideal weight. The terminalized mesocaval shunt created two separate sectors of the splanchic flow allowing: a) at least 50% reduction of portal flow into the graft which was thus exposed to hemodynamic conditions tolerable for a small-for-size graft with 24% of the ideal weight; b) maintained gastroduodenosplenic pancreatic flow to a functional graft thus avoiding potential disorders related to portosystemic shunts; c) supply of trophic and mitogenic factors via the duodenopancreatic flow enabling rapid graft regeneration.

Application of these results to human transplant recipients might allow use of small-sized orthotopic left lobe transplants (split liver or living donor transplantation) with a terminalized mesocaval shunt, thus increasing the possibilities for liver transplantation in adults.

ACKNOWLEDGEMENTS - The authors thank APICIL-ARCIL, Foundation B, and Novartis for their support.

REFERENCES

Hemodynamic tolerance and rapid hypertrophy of a hepatic graft corresponding to less than 30% of the ideal mass in pigs


