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Auxiliary liver transplantation: how to improve regeneration of the native liver by surgery

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Abstract The technical factors which could influence regeneration of the native liver (NL) in auxiliary liver transplantation (ALT) for fulminant hepatic failure (FHF) are not well known. We studied NL regeneration according to the location of graft anastomosis in the recipient's portal system (superior mesenteric vein versus portal vein), and graft weight (50% reduced-size versus full-size graft) in a rat model of ALT with 80% reduction of the NL, and graft arterialization. NL regeneration was significantly more obvious when the graft was anastomosed on the recipient's superior mesenteric vein, thus establishing venous flow to the NL from the pancreas, the spleen, and the stomach, and when a full-size graft was used. The influence of portal venous flow on NL regeneration, assessed by [^{3}H]-thymidine incorporation, was measurable as early as day 2. Both technical variables in combination resulted in significantly greater regeneration (ratio weight of NL/body weight at day 30: 2.32 ± 0.68% versus 1.21 ± 0.63% respectively, $P = 0.02$). Early preservation of portal flow to the NL is advisable to maximize NL regeneration in ALT. In any case, this regeneration is not impeded by the use of large auxiliary grafts.

Key words Liver transplantation, rat · Auxiliary liver transplantation, rat · Liver regeneration, rat

Abbreviations

ALT Auxiliary liver transplantation · *BW* Body weight · *FHF* Fulminant hepatic failure · *NL* Native liver · *OLT* Orthotopic liver transplantation

Introduction

Orthotopic liver transplantation (OLT) is a life-saving procedure in patients with fulminant hepatic failure (FHF) [1]. However, this technique does not take into account the ability of the native liver (NL) to regenerate, with minimal – and even lack of – sequelae, even if hepatocytes may have undergone complete necrosis. Thus, auxiliary liver transplantation (ALT) has been suggested as an alternative to OLT to provide temporary support. Once complete regeneration of the NL has taken place, immunosuppression is stopped and the graft can be removed [4]. A recent European study comprising 30 patients has shown that this aim can be achieved in up to 68% of patients undergoing ALT for FHF [5]. The ALT technique is less standardized than OLT, as the early and later roles of the auxiliary graft are contradictory in some respects. It is important to initially provide the recipient with a graft which is as large as possible, to rapidly restore liver function and reverse cerebral edema [2,

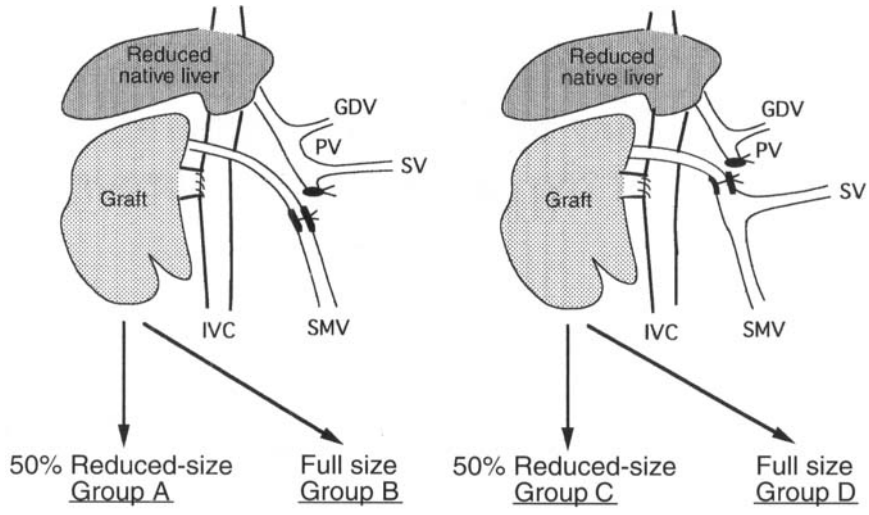

Fig. 1 Technique of auxiliary liver transplantation. *Left* The auxiliary graft is anastomosed to the superior mesenteric vein (*SMV*) and the native liver is perfused by the gastro-duodenal vein (*GDV*) and the splenic vein (*SV*) (*groups A&B*). *Right* The auxiliary graft is anastomosed to the portal vein (*PV*) and the native liver is perfused by the gastro-duodenal vein alone (*groups C&D*). In all groups, the native liver is 80% reduced and the venous drainage of the graft is restored by an end-to-side anastomosis onto the recipient inferior vena cava (*IVC*). The graft artery is reconstructed by end-to-side aorto-aortic anastomosis

4, 14]. In the long term however, a large graft might prevent NL regeneration because of both the presence of a large total hepatic mass [10] and the diversion of most of the portal blood flow away from the NL [21]. To address this issue, we studied NL regeneration in a rat model of ALT with reduced or full-size syngeneic arterialized grafts, anastomosed onto the recipient's superior mesenteric vein or portal vein.

Materials and methods

Experimental design

To determine the effect of graft-size and the location of portal anastomosis to the native liver (NL) regeneration, we performed an 80% hepatectomy of the NL, and compared NL regeneration in 4 groups (Fig. 1):

- Group A = 50% reduced-size auxiliary graft, anastomosed to the recipient's superior mesenteric vein.
- Group B = full-size auxiliary graft, anastomosed to the recipient's superior mesenteric vein.
- Group C = 50% reduced-size auxiliary graft, anastomosed to the recipient's portal vein.
- Group D = full-size auxiliary graft, anastomosed to the recipient's portal vein.

Male Lewis rats (Iffa-Credo, l'Arbresle, France) weighing 280–320 g, were used as donors and recipients. They were housed and maintained under controlled conditions in our laboratory animal facilities. Commercial pellets and water were available ad libitum.

Donor operation

Livers were harvested under ether anaesthesia as previously described [22]. After intravenous injection of heparin (200 IU), the liver was perfused with 20 ml of cold saline solution (4°C) through cannulae placed in the portal vein. The infrahepatic portion of the inferior vein cava and the portal vein were divided. The suprahepatic vena cava was ligated and divided above the diaphragm. The common bile duct was divided at the upper edge of the pancreas, and the accessory left hepatic artery was ligated and divided. The common hepatic artery was traced to the celiac trunk which was harvested with an attached cuff of the aorta. The graft was weighed and the standard liver volume was defined as the means of the ratios of liver weight/body weight, calculated for each donor. The graft was put in a container with 4°C cold saline, and a polyethylene cuff (1.6 mm inner and 2.1 mm outer diameter) was attached to the portal vein. In half of the donors, a 50% reduction was performed by means of resection of the left lateral lobe, the caudate lobe (anterior and posterior segments) and the left portion of the median lobe, in which caval stenosis was carefully avoided [18]. The donor and recipient animals were prepared simultaneously to reduce the transplant ischemia time.

Recipient operation

Under ether anaesthesia, an 80% reduction in NL size was performed without clamping, by removing all lobes except the superior part of the right lobe (segment VII). A 90% hepatectomy was not performed because of the difficulties of biopsy of the remaining caudate lobe in early follow-up after ALT. The actual rate of NL reduction was calculated for each rat according to the formula: rate of reduction = weight of resected segments/standard liver volume.

The auxiliary graft was placed heterotopically in the right paravertebral gutter. Graft outflow was re-established by anastomosing end-to-side (8/0 running suture) the infrahepatic vena cava of the graft to the recipient's vena cava at the level of the re-

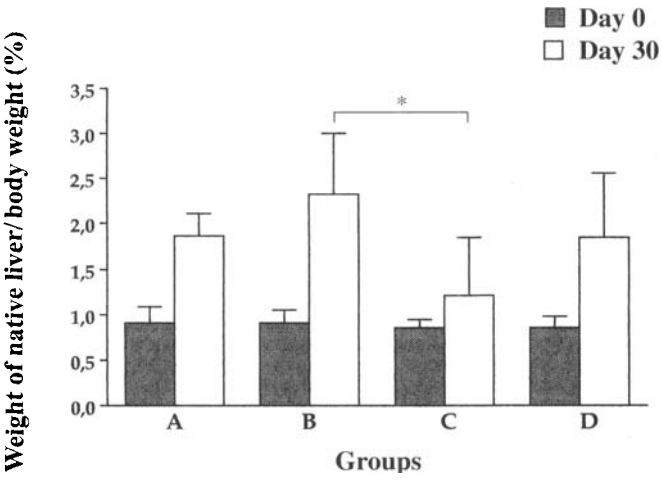

Fig. 2 Ratio weight of native liver/body weight at day 0 (auxiliary liver transplantation) and day 30 (sacrifice). Data are means ± SD ($n = 6$ in each group). *$P = 0.012$

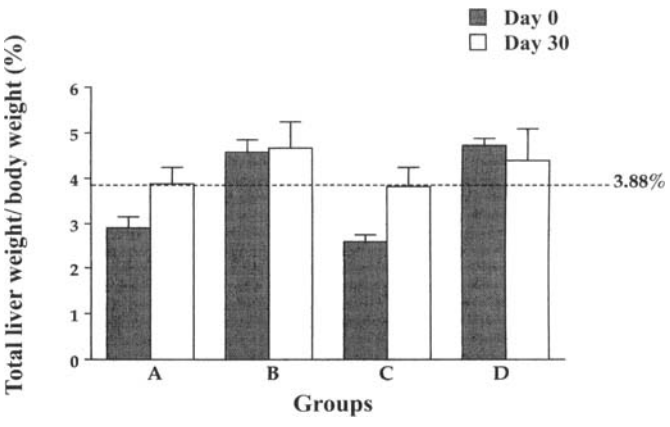

Fig. 3 Ratio total (native liver + auxiliary graft) liver weight/body weight at day 0 (auxiliary liver transplantation) and day 30 (sacrifice). Data are means ± SD ($n = 6$ in each group). The value 3.88 % is the mean of the ratios weight of native liver/body weight in the donors

nal veins, under transient lateral clamping. The graft portal vein was anastomosed either to the recipient's superior mesenteric vein or to the recipient's portal vein, in an end-to-end fashion, using the cuff technique (Fig. 1). If the recipient's portal vein was used, portal flow to the NL was preserved via the gastro-duodenal vein. All auxiliary grafts were rearterialized by anastomosing the donor aorta end-to-side (8/0 running suture) to the recipient's infrarenal aorta. Bile flow was restored by insertion of the intubated donor bile duct into the duodenum [22].

Postoperative care

Rats were given 4 ml of warm saline solution through the penile vein after graft revascularization. Postoperatively, rats were placed in individual cages. In surviving rats ($n = 6$ in each group), needle biopsies of the NL (segment VII) were taken under light ether anaesthesia at days 2 and 4. At day 30, all rats were killed by intravenous pentobarbital overdose. The "Principles of laboratory animal care" (NIH publication No.86–23, 1985) were followed for the animal experiments.

Samples and measurements

Samples and measurements were performed in 24 rats ($n = 6$ in each group). Biopsies of the NL at days 2 and 4 (3 rats in each group at each date) were performed 1 h after an intravenous injection of 0.6 μCi [^{3}H]-thymidine/g of body weight (BW). Days 2 and 4 were chosen because the regeneration peak after liver transplantation in rats is delayed, compared with partial hepatectomy [3, 13]. Paraffin sections of liver tissue samples were covered with an autohistoradiography emulsion (emulsion K5, Ilford, Saint Priest, France) and developed after 2 weeks.

At day 30 the rats were sacrificed one hour after withdrawal of 1 ml of blood for liver tests (bilirubine, factor II, factor VII + X) and an intravenous injection of bromodeoxyuridine (BrdU, 100 mg/kg of BW). Patency of all anastomoses was assessed and confirmed. Both the auxiliary graft and NL were excised, cleaned of gross adhesions, exsanguinated, and weighed. The rate of variation of the weight of the NL between day 0 (ALT) and day 30 was calculated, as well as the ratios, weight of NL/BW, and total liver weight/BW (total liver weight = weight of NL + weight of graft) at days 0 and 30. Paraffin sections of liver tissue samples were immunostained with a murine monoclonal antibody against BrdU (Novocastra, Newcastle upon Tyne, United Kingdom). Labelling index (S-phase index) was scored on biopsies at days 2, 4, and 30 in 50 microscopic fields representing 100 hepatocytes each.

Statistical analysis

Results are expressed as means ± standard deviation. Statistical analysis was performed with one or two way ANOVAS when appropriate. Values of $P < 0.05$ were considered significant.

Results

Surgical procedure

The surgical mortality was 20 % (6/30). All postoperative deaths (one or two in each group) occurred within the first 24 postoperative hours due to haemorrhage. In donors, the standard liver weight (mean of ratios liver weight/BW; $n = 24$) was 3.88 ± 0.24 %. The actual ratio of graft reduction (weight of resected parenchyma/total weight of donor liver) in groups A and C was 47.2 ± 3.8 % ($n = 12$) (designated ratio: 50 %).

In recipients, there was no difference between the groups for BW, weight of NL/BW (Fig. 2), cold ischaemia time, and duration of recipient procedure. The actual ratio of NL reduction (weight of resected parenchyma/3.88 % of BW) was 78.7 ± 3.5 % ($n = 24$) (designated ratio: 80 %). After ALT, the ratio of total liver weight/BW was 2.88 ± 0.25 % in group A, 4.58 ± 0.28 % in group B, 2.68 ± 0.15 % in group C, and 4.73 ± 0.16 % in group D respectively (Fig. 3).

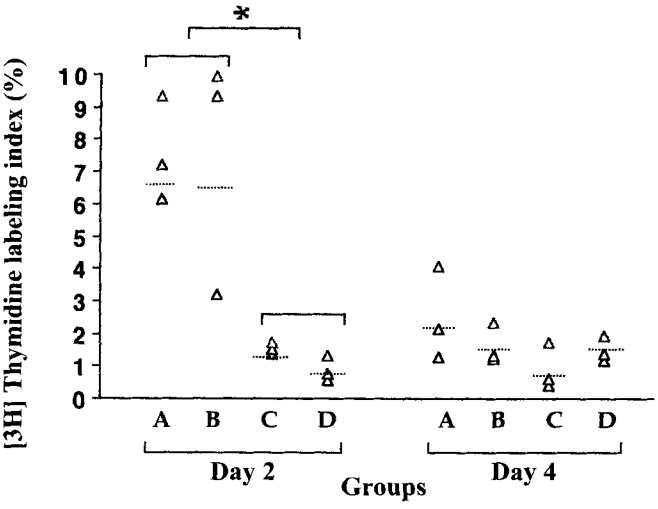

Fig. 4 Distribution of [3H]-thymidine labelling indexes in the native liver at day 2 and day 4 following auxiliary liver transplantation. *$P = 0.0007$

Regeneration of the native liver

At day 2, [^{3}H]-thymidine incorporation in the NL was significantly higher when the auxiliary graft was anastomosed to the recipient's superior mesenteric vein (groups A and B versus groups C and D) (Fig. 4). No significant histological changes were noted on native liver tissue samples. On the other hand, [^{3}H]-thymidine incorporation did not change in relation to the graft size (Fig. 4). This difference resolved at day 4.

At day 30, no difference was noted between groups for liver function tests, which were normal in all cases. The labelling index of S-phase cells was within normal values (< 0.02 %) in all rats. Pathological examination of liver tissue samples showed only mild steatosis in most cases. The rate of variation of the NL weight between day 0 and day 30 was + 178 ± 58 % in group A, + 212 ± 123 % in group B, + 96 ± 81 % in group C, and + 178 ± 127 % in group D respectively.

Regeneration of the NL was significantly more marked when the auxiliary graft was anastomosed to the recipient's superior mesenteric vein (groups A and B versus groups C and D) and when a full-size graft was used (groups B and D versus groups A and C) (Fig. 2). The ratio between weight of NL/BW at day 30 was higher in groups A + B than in groups C + D (2.10 ± 0.54 versus 1.53 ± 0.69, $P = 0.025$). This ratio was higher in groups B + D than in groups A + C (2.09 ± 0.71 versus 1.54 ± 0.54, $P = 0.028$). In groups B and C, which used opposite techniques, the ratio between weight of NL/BW was 2.32 ± 0.68 % and 1.21 ± 0.63 % respectively ($P = 0.012$). The ratio between total liver weight/BW was normalized in groups A (3.87 ± 0.37 %) and C (3.82 ± 0.41 %), and was still above the normal value in groups B (4.65 ± 0.60 %) and D (4.56 ± 0.63 %) (Fig. 3).

Discussion

The results of the present study confirm that the way portal flow is shared between the auxiliary graft and the NL is an essential determinant of NL regeneration in ALT. Surprisingly, we can show that a large auxiliary graft can also enhance regeneration of the NL. The influence of each technical variable is independent from each other. Our study suggests that the surgical technique can increase the possibility of NL regeneration in auxiliary liver transplantation (ALT) for fulminant hepatic failure (FHF).

Functional competition between the NL and the auxiliary graft has been emphasized in several clinical studies [2, 4, 5, 16, 20, 21]. These studies mainly focus on the influence of underlying liver disease and graft rejection [2, 4, 5]. None of these studies come to definite conclusions about the best way to share portal blood flow in the ALT for FHF. Furthermore, in the only two clinical series of ALT for FHF, poor portal blood flow is not mentioned as a possible cause of non-regeneration of the NL [2, 5]. In ALT for chronic liver disease, some authors suggest that interruption of portal flow to the NL may ensure good graft function, but this is contradictory to the long-term aim of ALT for FHF [21]. Experimentally, in a rat model of ALT, preservation of the portal flow to the NL and perfusion of the graft by the caval flow is not a good option, since this technique results in graft atrophy within 2 weeks [22]. Other experimental studies focus on the share of portal flow in ALT. In a study by Hess et al., decreased portal blood flow into the NL is associated with maintained, or a decrease in, NL weight. This work mainly studies graft regeneration in relation to a functional handicap of the NL, which is a situation far from clinical ALT [7]. Yu et al. demonstrate variations in NL atrophy in relation to the location of the graft anastomosis in the recipient's portal system but in this study all grafts and NL are full-sized and are for the greater part embolized with islet isografts prior to ALT [22]. In allogeneic ALT in pigs, Nagashima at al. [17] demonstrate that a 50 % graft progressively atrophies with parenchymal necrosis when there is no banding on the NL portal vein. On the other hand, portal banding of the NL results in good graft regeneration [17]. In the present study, the larger splanchnic territory perfusing the NL, when the graft was anastomosed to the recipient's superior mesenteric vein, resulted in better regeneration of the NL, whatever the graft size. This occurs early in the post-operative period, as demonstrated by the greater index of S-phase cells of the NL at day 2 in groups A and B. Although we did not perform hemodynamic measurements, we can estimate

that the proportion of portal blood flow to the NL is approximately one third in groups A and B, and one fifth in groups C and D, as demonstrated previously in hemodynamic studies in the rat [19]. We can conclude that precise assessment of portal flow in both graft and NL must be performed peroperatively and early in the postoperative period to maximize chances of NL regeneration. Peroperative measurement of portal pressure in both graft and NL portal vein, adequate banding on the graft portal vein, and Doppler ultrasonography, could be useful for this purpose [20]. However, we can not assume from our results that pancreatic venous blood has more hepatotrophic properties than blood from the superior mesenteric vein. Pancreatic hormones, e.g. insuline and glucagon, have been supposed to be the most important hepatotrophic factors [9]. In fact, hepatotropic properties of the blood from the superior mesenteric vein are likely, considering results of experimental studies [22] and synthesis of other hepatotrophic factors – including Hepatocyte Growth Factor – by the intestinal mucosa [9, 15]. Because the lack of correlation of liver regeneration to serum levels of these factors [6, 9] and a possible paracrine mechanism of up-regulation for Hepatocyte Growth Factor [9, 15], we did not perform serum measurement.

One original finding in this study is the influence of graft weight on NL regeneration. In clinical ALT, the graft can be reduced to a right liver (approximately 65% of the whole liver volume), a left liver (35%) or a left lobe (20%) [2, 4, 12]. In ALT for FHF, the necessity of quick restoration of liver function and reversal of cerebral oedema argues for the use of the right liver [2, 4, 14]. The drawbacks of large auxiliary grafts are increased technical difficulty due to the risk of graft compression during abdominal closure, and a theoretical risk of impaired NL regeneration, since standard liver weight is regulated by BW [2, 9]. We observed the opposite result, since NL regeneration was better with full-size grafts than with 50%-size grafts independent of the site of portal anastomosis. There is no clear explanation for this unexpected finding. One hypothesis could be secretion of systemic hepatotrophic factors in proportion to the amount of graft parenchyma [9]. Our results also argue for the use of large grafts in ALT for FHF. However, no definite conclusion can be drawn, because our model differs from clinical ALT for FHF, due to the lack of hepatocytes damage on the NL.

Restoration of standard liver-weight is as commonly observed after OLT [10, 12] as after partial hepatectomy [6, 9]. In OLT with an initial graft weight less than the standard liver weight, there is a progressive increase in graft weight towards 100% of the standard liver weight [10, 12]. In our model of ALT, we observed restoration of total liver weight after using 50% size grafts. On the other hand, excess total liver weight clearly persisted in groups B and D at day 30. It is commonly accepted that excess liver weight is eliminated after liver transplantation. To our knowledge, only one clinical case has been documented in a child that underwent OLT with a graft from his father. The graft represented 192% of the standard liver weight at OLT, and 142% three months later, due to an increase in the patient's BW [11]. In fact, OLT with grafts larger than the standard liver weight are rarely performed, due the risk of graft compression or worsening of hemodynamics during abdominal closure [8]. Yu et al. performed several combinations of ALT in rats with an initial excess total liver weight in all cases [22]. They observed that the ratio of total liver weight/BW was normalized 12 weeks after ALT when one of the two livers was deprived of portal flow, while a 12% excess liver weight persisted at this date when portal flow was shared [22]. Our results, as those of Yu et al., suggest that downregulation of the standard liver weight is slower than up-regulation and does not clearly impede NL regeneration in ALT.

In conclusion, we have developed an experimental rat model of ALT including an 80% reduction in the NL and arterialized auxiliary grafts of varying sizes (full or 50% reduced), which were anastomosed to either the recipient's portal vein or to the superior mesenteric vein. Our study confirms that portal flow to the NL should be preserved as early as possible, and demonstrates that in any case, use of large auxiliary grafts does not impede the regeneration of the native liver. To determine clinical applications in the field of ALT for FHF, these results should be confirmed in a model including ischemic or toxic damage of hepatocytes on the NL.

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