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Restoration of liver mass after partial hepatectomy – implications for living donor liver transplantation

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Summary

In living donor liver transplantation, the recipient liver undergoes more rapid regeneration than the remnant liver in the donor. In this study we investigated the factors which may be responsible for the difference in the regenerative response between the donor and the recipient.

Long Evans rats were subjected to either partial hepatectomy (PH) or sham operation (SH) and were treated with liver cytosol (C) and cyclosporine (Cy). The rats were sacrificed at 24, 48, 72 and 96 hours and 1 and 2 weeks postoperatively. The livers were removed to determine the liver weight/body weight (LW/BW) ratio and the mitotic index.

The mitotic index, serum aspartate transferase (AST) and serum alanine transferase (ALT), although unchanged in the SH groups, were increased in the rats treated with PH + C + Cy, and were greater than after PH only. However LW/BW ratios increased after PH but had returned to preoperative levels by 2 weeks. The changes in LW/BW ratio were not modified by the cytosol or cyclosporine.

Liver transplantation is the treatment of choice for most patients with end-stage liver disease and is performed on a routine basis in most major centres throughout the world. Liver transplantation, unfortunately, has become a victim of its own success in that the supply of donor organs has not been able to keep up with the demand. The critical shortage of donor livers for transplantation has been addressed in several ways, including the use of reduced size adult livers for paediatric recipients, the use of split liver transplants, and more recently the use of living donor liver transplants.[1-3] The latter strategies have been the result of a better understanding of the anatomy of the liver and knowledge of the latent capacity of the liver to undergo regeneration after partial hepatectomy.

Liver regeneration has been extensively studied and several factors have been shown to modify the regenerative response.[4,5] For example, cyclosporine has been shown to potentiate the regenerative response after partial hepatectomy.[6-9] In addition, the regenerating liver itself has also been shown to contain hepatotrophic factors which potentiate liver regeneration.[10-12] However the precise factors which initiate and terminate the regenerative response remain unre solved.[13-16]

Liver regeneration in the recipient and in the donor after living donor liver transplantation has been studied to a limited extent using computed tomography (CT) scan estimation of liver size.[17,18] Several studies have shown that the donor liver takes longer to restore liver mass than the transplanted liver in the recipient. In fact, liver mass in the donor had still not been restored to preoperative size by 1 year after surgery. Several factors may be responsible for this discrepancy in the regenerative response between the recipient and the donor. Firstly, the recipient has high levels of circulating hepatotrophic factors because of the liver disease. Secondly, the recipient also receives cyclosporine, which is known to be hepatotrophic, in the post-transplant period.

The aim of this study was to investigate how long it took for liver mass to be restored after partial hepatectomy in rats and to investigate the effect of the hepatotrophic factors on the regenerative response.

Materials and methods

Adult male Long Evans rats weighing 200 - 250 g were maintained under standard environmental conditions and allowed *ad libitum* access to a standard rat pellet diet and water. Following an equilibration period, the rats were subjected to either standard two-thirds partial hepatectomy (PH) or sham operation (SH). All surgical procedures were performed under light ether anaesthesia between 08h00 and 11h00. The animals were randomly allocated to the following treatment groups: (*i*) group 1 – partial hepatectomy (PH) (N = 30); (*ii*) group 2 – partial hepatectomy + cyclosporine + liver cytosol (PH + Cy + C) (N = 30); (*iii*) group 3 – sham

operation (SH) (N = 30); and (*iv*) group 4 – sham operation + cyclosporine + liver cytosol (SH + Cy + C) (N = 30).

Surgical procedures

A standard two-thirds partial hepatectomy was performed via a midline laparotomy and involved removal of the left lateral and middle lobes of the liver. Sham operation consisted of a midline laparotomy and gentle manipulation of the liver.

Preparation of liver cytosol

A separate group of 5 animals was subjected to two-thirds partial hepatectomy and sacrificed 24 hours postoperatively. The remnant livers were removed, homogenised and subjected to ultracentrifugation at 105 000 *g*. The supernatant served as the liver cytosol.

Injection of cytosol and cyclosporine

The animals in groups 2 and 4 received a daily intraperitoneal injection of liver cytosol at a dose of 0.685 mg protein in 5 microlitres. These animals also received cyclosporine 10 mg/kg orally.

Sacrifice

Five animals from each of the above groups were sacrificed at 24, 48, 72 and 96 hours and 1 and 2 weeks postoperatively. The animals were exsanguinated under ether anaesthesia via a midline laparotomy and the liver remnant removed.

Investigations

The blood specimens were used to measure the liver function tests. The liver remnants were used to measure the liver weight/body weight ratio and for histological examination to determine the mitotic indices.

Results

The changes in serum alanine transferase (ALT) levels are shown in Fig. 1. The serum ALT levels remained unchanged after sham operation (group 3). There was a significant increase in serum ALT at 24 hours after partial hepatectomy (group 1). Thereafter there was a gradual decrease in ALT and a return to normal by 96 hours postoperatively. Serum ALT levels were significantly higher at 24 hours in the animals subjected to partial hepatectomy and cytosol infusion (group 2). The serum ALT levels in the animals subjected to sham operation with an injection of cyclosporine and cytosol (group 4) were also slightly higher than after sham operation only (group 3). The higher serum ALT levels in the animals in groups 2 and 4 compared with groups 1 and 2 were probably related to the infusion of the liver cytosol.

The changes in the serum AST levels are shown in Fig. 2. The changes in serum aspartate transferase (AST) levels were similar to the changes in the serum ALT.

The changes in the mitotic indices in the four groups are shown in Fig. 3. There was a significant increase in mitotic indices after partial hepatectomy at 24 hours and a further increase at 48 hours postoperatively. Thereafter the mitotic indices decreased to preoperative levels by 2 weeks. The mitotic indices in the animals subjected to PH + Cy + C

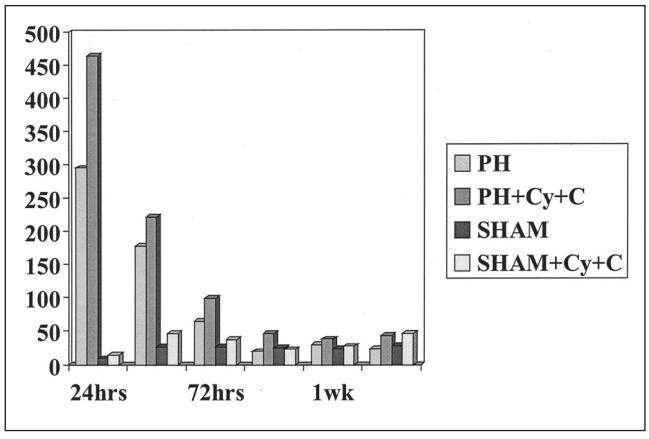

Fig. 1. Changes in serum alanine transferase (ALT) after standard two-thirds partial hepatectomy and administration of cyclosporin and liver cytosol.

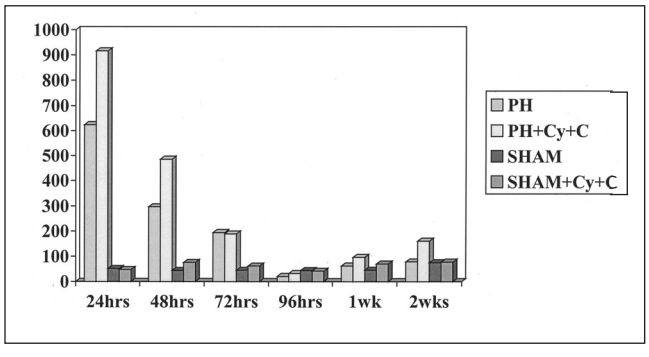

Fig. 2. Changes in serum aspartate transferase (AST) after standard two-thirds partial hepatectomy and administration of cyclosporin and liver cytosol.

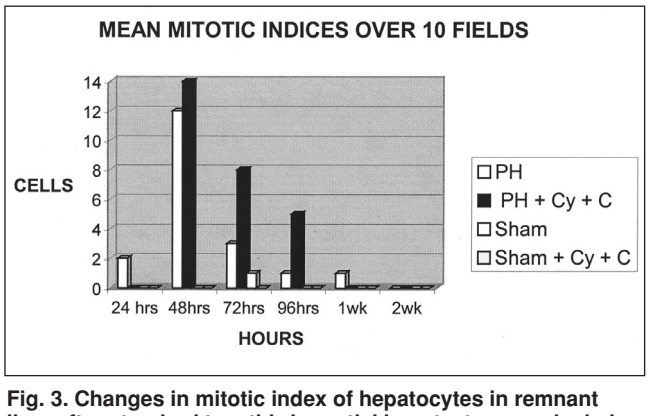

Fig. 3. Changes in mitotic index of hepatocytes in remnant liver after standard two-thirds partial hepatectomy and administration of cyclosporin and liver cytosol.

(group 2) were significantly higher than in the animals in group 1. There were no mitotic figures seen in the animals subjected to sham operation.

The changes in the liver weight to body weight ratios in the animals studied are shown in Fig. 4. There was a significant reduction in the liver weight to body weight ratio at 24 hours after partial hepatectomy. This was compatible with a two-thirds partial hepatectomy. Thereafter the liver weight to body weight ratios in the animals in groups 1 and 2 increased steadily, but had still not reached preoperative levels by 2 weeks after partial hepatectomy. Interestingly, the liver weight to body weight ratios in the animals subjected to

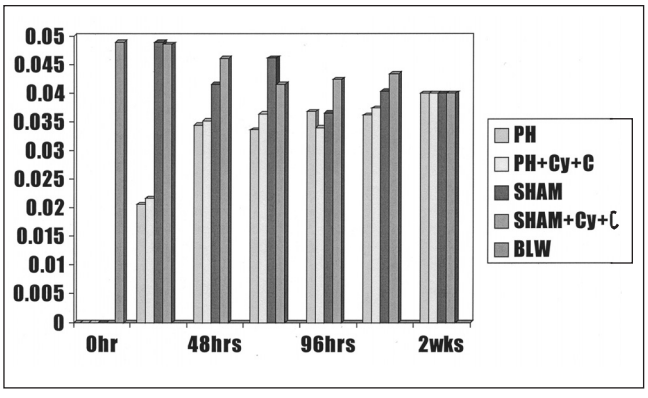

Fig. 4. Changes in liver weight to body weight ratio in the groups compared with baseline liver weight.

sham operation decreased slightly over the 2 weeks. Treatment with liver cytosol or cyclosporine did not influence the liver weight to body weight ratio.

Discussion

Living donor liver transplantation is now performed routinely in most major centres throughout the world. The regenerative response in the remnant liver in the donor and in the transplanted liver in the recipient has been studied previously.[1,2] It has been noted that restoration of liver mass in the donor takes longer than in the recipient, and that the liver volume does not return to preoperative size by 12 months after the surgery. Several factors in the recipient may account for the discrepancy in the regenerative response compared with the donor.

In this study we noted that the liver mass had not yet returned to preoperative levels by 2 weeks after partial hepatectomy in rats. The peak regenerative response, using DNA synthesis and mitotic index as markers of liver regeneration, is known to occur during the first 24 to 48 hours after partial hepatectomy.[7] Most studies have been limited to the first postoperative week, and the above markers have usually returned to normal by then. Restoration of liver mass is generally not used as an endpoint of liver regeneration.

Liver mass is obviously a very crude estimation and is influenced by water and fat content. These factors were not taken into consideration in these studies. However, the histological evaluation of the livers did not show any evidence of increased fat in the livers. In the clinical studies of liver transplant donors and recipients, liver mass has been estimated using CT scan calculations, which are also relatively inaccurate.[17]

The recipient presumably has high levels of hepatic growth factors in the circulation as a result of the liver disease. To simulate this, we infused liver cytosol from regenerating livers into animals after partial hepatectomy or sham operation. The liver cytosol did appear to modify the regenerative response after partial hepatectomy as indicated by the increased mitotic index. However, liver cytosol did not initiate a regenerative response after sham operation. Furthermore, liver cytosol did not modify the restoration of liver mass after partial hepatectomy.

Cyclosporine has also been shown to potentiate the regenerative response after partial hepatectomy.[6-9] In this study the animals receiving cyclosporine after partial hepatectomy had a greater mitotic index level after partial hepatectomy. However, the restoration of liver mass was not modified by the addition of cyclosporine.

In summary, therefore, liver mass in this study was not restored by 2 weeks after partial hepatectomy even though the mitotic index had returned to normal after 48 - 72 hours. Hepatotrophic factors and cyclosporine, which are thought to be responsible for the more rapid growth of the liver in the recipient compared with the donor in living donor liver transplantation, did not modify the restoration of liver mass.

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