World Journal of Medical Sciences 11 (4): 468-472, 2014
ISSN 1817-3055

DOI: 10.5829/idosi.wjms.2014.11.4.8362

Histological Study of Liver Regeneration Following Partial Hepatectomy and Total Splenectomy

[1]Kalbaza Ahmed Yacine, [1]Amara Karim, [1]Hemida Houari,
[1]Benchohra Mokhtar and [2]Melizi Mohamed

[1]Institute of Veterinary Sciences, Ibn-khaldoun University, Tiaret (14000), Algeria
[2]Institute of Veterinary and Agronomic Sciences, Hadj Lakhdar University, Batna (05000), Algeria

Abstract: "Small for size" syndrome is a major problem in hepatic surgery. The aim of this study is to investigate the effect of total splenectomy on liver regeneration following partial hepatectomy in rabbits. Animals were divided into two groups, 6 each: "Hx": hepatic resection and "Hx+Sp": hepatic resection and total splenectomy. Three days after skin closure, animals in the two groups were sacrificed and the remnant liver tissue was harvested to perform a histopathological examination. Animals in the "Hx+Sp" group have shown a pronounced liver regeneration signs compared to those of the "Hx" group.

Key words: Small For Size Syndrome · Liver Regeneration · Hepatic Resection · Total Splenectomy · Rabbits

INTRODUCTION

Continuous refinements in surgical technique have resulted in better performance of extended hepatic resections and transplantation of partial liver grafts procured from deceased and living donors [1, 2]. However, the minimal amount of liver mass that can ensure a proper balance between liver regeneration, liver function and metabolic demands is still debated [3, 4]. "Small for size" syndrome is a clinical syndrome described after liver transplantation or extended hepatic resection. This postoperative liver dysfunction is characterized by prolonged cholestasis, coagulopathy, persistent portal hypertension and it is associated with high morbidity and mortality rates [5].

In order to avoid the occurrence of "Small for size" syndrome after extended hepatectomy and liver transplantation, a minimal functional liver mass is recommended: remnant liver should be 25–30% of the standard liver volume in patients with healthy livers, whereas in presence of restricted liver function this percentage should be increased to 40–50% [6, 7]. In addition to liver mass, several factors are involved in the pathogenesis of "Small for size" syndrome, such as the underlying liver disease, the severity of liver disease and suboptimal liver vascular outflow [8].

Splenectomy may have beneficial effect after living donor transplantation and massive hepatectomy. However, the exact mechanism is still unclear, especially since the remnant liver tissue has never been subject of histopathological examination. With the aim to clarify the relationship between total splenectomy and "Small for size" syndrome, we hypothesized that splenectomy accelerates liver cell proliferation and we compared the signs of hepatocytes proliferation after extended hepatecomy and its combination with total splenectomy in rabbits.

MATERIALS AND METHODS

Experimental Design: The study was carried out by using twelve male local rabbits with a mean weight of 1.5 - 2 kg which were randomly assigned into two groups as follow: Hepatic resection "Hx" (n = 6) and hepatic resection with splenectomy "Hx+Sp" (n = 6). Prior to any procedure, animals were acclimatized for 3 days under standardized laboratory conditions in a temperature-controlled room. Rabbits were individually housed, had free access to standard laboratory food and water and were subjected to a 12 h light/dark cycle per day.

All rabbits in the two groups were sacrificed at day 3 after surgery and remnant liver tissue was harvested to perform histopathological examination.

Corresponding Author: Kalbaza Ahmed Yacine, Institute of Veterinary Sciences, Ibn-khaldoun University, Tiaret (14000), Algeria.

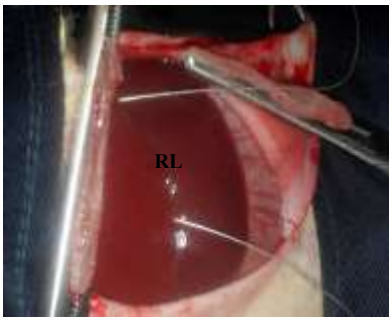

Fig. 1: Ligature of the liver lobe RL: resected lobe

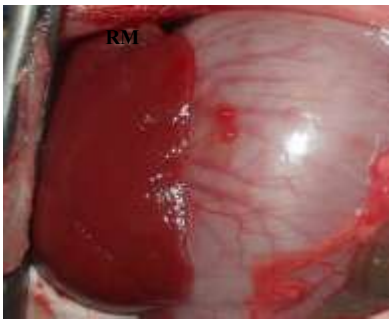

Fig. 2: Liver lobe resection RM: resection margin

Fig. 3: Ligation and section of the splenic vessels S: Spleen

Surgical Procedures: Hepatic resection was performed as described previously by Higgins and Anderson [9]. The rabbits were anesthetized by 0.8 mg/kg intramuscular Acepromazine, 0.05 mg/kg Buprenorphine and 40 mg/kg of Ketamine then a midline laparotomy was performed. After opening the upper abdomen and exposure of the liver, the lobe to be resected was gently lifted while a 3-0 silk suture tie was placed underneath it and positioned as proximal to the origin of the lobe as possible (Fig. 1). The two ends of the suture were tied over the top of the lobe at its base near the inferior vena cava. Three knots were tied and dissecting scissors were used to cut the tied lobe just distal to the suture (Fig. 2). Then the abdominal wall was reapproximated with a running 3-0 Polyglactin[910] suture and the skin was closed with a running 2-0 polyamide suture.

After exactly the same surgery, rabbits in the "Hx+Sp" groups received a splenectomy, in which the splenic artery and vein were ligated with 3-0 silk (Fig. 3) and the spleen was removed from the abdomen.

Histopathological Examination: Three days after skin closure, animals in the two groups were sacrificed and the remnant liver tissue was removed. The livers were fixed in 10% buffered formaldehyde, dehydrated in graded ethanol, treated in xylene and infiltrated and embedded in paraffin. Coronal sections (5ìm) were cut using a paraffin microtome, mounted on coated slides and then dried at 37°C overnight on a hot plate. An average of two sections were prepared for each liver

RESULTS

Histopathological evaluation of liver regeneration was based on various morphological changes which were predominated by the number of binucleated hepatocytes and mitotic figures and thickening of liver cell plates.

The liver tissue remaining after partial hepatectomy showed cell proliferation characterized by the appearance of the most important signs of liver regeneration, which can be summarized in a few binucleated hepatocytes, some mitotic figures and thickening of some liver cell plates. (Fig. 4), however, no ductular reaction was observed.

The observation of liver tissue remaining after partial hepatectomy showed that these changes have been more prominent in animals of "Hx+Sp group. The number of mitotic figures and binucleated cells was higher after removal of the spleen. This important cell proliferation resulted in thickening of liver cell plates with the arrangement of more than two hepatocytes in the same plate, leading to a narrowing of the lumen of the sinusoids (Fig. 5).

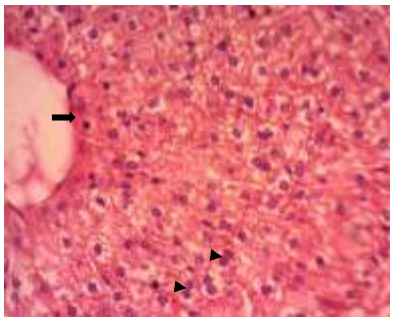

Fig. 4: Remnant liver tissue after partial hepatectomy showing some binucleated hepatocytes (arrow head) and double-cell plates (arrow); 400X; H&E.

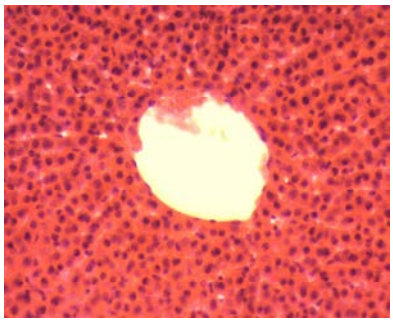

Fig. 5: Liver tissue section of "Hx+Sp" rabbits showing prominent mitotic figures and numerous binucleated hepatocytes; 400X; H&E.

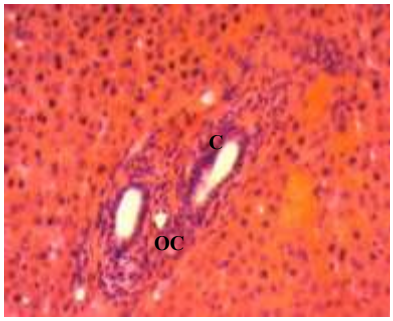

Fig. 6: Ductular reaction with proliferating chloangiocytes and oval cells, rabbit; 400X; H&E.
C: Cholangiocytes
OC: Oval cells

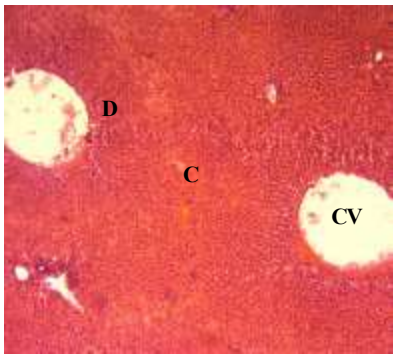

Fig. 7: Liver section representing enlarged central veins and irregular distribution of cell proliferation creating clear and dark appearance of hepatic pranchyma, rabbit; 100X; H&E.
D: Dark area
C: Clear area
CV: Centrilobular vein

Other morphological changes were observed in splenectomized animals such as ductular reaction which consists of cholangiocytes and oval cells proliferation (Fig. 6) and the expansion of central veins (Fig. 7).

The increase in the number of mitotic figures and binucleated hepatocytes was much higher in the centrilobular area, resulting in the creation of light and dark areas (Fig. 7).

DISCUSSION

Progress in liver surgery field resulted in achieving better extended liver resection and transplantation of partial grafts obtained from living or deceased donors [10, 1]. Loss of liver tissue is the most potent stimulus for hepatic regeneration [11]. This vital process aiming to restore liver mass is hampered by the "Small for size" syndrome, a clinical manifestation described after liver transplantation or extensive hepatectomy, which leads to a hepatic dysfunction characterized by prolonged cholestasis, persistent portal hypertension, coagulopathies and high morbidity and mortality [5].

Minimal hepatic mass that can ensure a good balance between liver functions and regeneration and metabolic demands is still controversial [4, 3]. Shoup *et al*. [6] and Kubota *et al*. [7] have described that the minimal mass to avoid the appearance of "Small for size" syndrome after extensive hepatectomy or liver transplantation should represent 25-30% of the standard liver volume of a healthy person, whereas in the presence of a liver disease, that percentage should be increased to 40-50%. The mechanism by which "Small for size" syndrome occurs is not fully explained and therapeutic strategies are very limited. However, preventing is essential and acceleration of liver regeneration in the early postoperative period appears as an attractive option [12].

The role of splenectomy in "Small for size" livers is under debate. On one hand, some surgeons believe that splenectomy should not be recommended with liver transplantation or major hepatectomy. Samimi *et al*. [13] found that splenectomy with orthotropic liver transplantation has a significantly higher patient mortality mainly due to septic complications. Neumann *et al*. [14] showed that splenectomy is a major risk factor for the development of opportunistic pneumonia caused by *Legionella pneumophila*, *Pneumocystis carinii*, *Aspergillus* species and *Cytomegalovirus* after liver transplantation. On the other hand, some reports indicated that splenectomy is effective in improving disorders of "Small for size" livers [12, 15] and remnant liver function after partial hepatectomy with total hepatic vascular exclusion [16].

Histopathology of liver regeneration after partial hepatectomy is well documented. However, no study has focused on the histopathological aspect of this regeneration after partial hepatectomy and total splenectomy. Our results are in correlation with those obtained by Dancygier [11], Castaing *et al.* [17] and Fausto *et al*. [18] indicating that signs of regeneration after partial hepatectomy include an increased cell proliferation and mitotic rate which is evidenced by thickening of liver cell plates with widespread double-cell plates which makes sinusoids more difficult to discern.

In our study, total splenectomy was in charge of the prominence of these signs which were more obvious and more numerous. The number of binucleated hepatocytes and mitotic figures were significantly higher in splenectomized animals, reflecting the important cell proliferation. Light and dark areas observed can be explained by Michalopoulos and DeFrances [19] works showing that cell proliferation begins around portal triads then progress to reach the hepatocytes located near the central veins in 48 hours.

Ductular reaction corresponding to cholangiocytes and oval cells proliferation accompanies a regenerative phenomenon after extensive hepatic parenchymal loss. In our study, this reaction was observed in the livers of animal who underwent total splenectomy and partial hepatectomy certifying that regeneration has interested hepatocytes and oval cells known as liver stem cell and a storage compartment [11].

In summary, total splenectomy accelerates liver regeneration in a rabbit model. The mechanism of this effect needs further researches to be clarified.

REFERENCES

1. Abu Hilan, M. and J.P. Lodge, 2000. Pushing back the frontiers of resectability in liver cancer surgery. European Journal of Surgical Oncology, 34: 272-80.
2. Foster, R., M. Zimmerman and J.F. Trotter, 2007. Expanding the donor options: marginal, living and split donors. Clinics in Liver Disease, 11: 417-29.
3. Mullin, E.J., M.S. Metcalfe and G.J. Maddern, 2005. How much liver resection is too much? American Journal of Surgery, 190: 87-97.
4. Kiuchi, T., K. Tanaka, T. Ito, F. Oike, Y. Ogura, Y. Fujimoto and K. Ogawa, 2003. Small for size graft in living donor liver transplantation: how far should we go? Liver Transplantation, 9(1): 29-35.
5. Tucker, O. and N. Heaton, 2005.The small for size liver syndrome. Current Opinion in Critical Care, 11: 150-5.
6. Shoup, M., M. Gonen, M. D'Angelica, W.R. Jarnagin, R.P. DeMatteao, L.H. Schwartz, S. Tuorto, L.H. Blumgart and Y. Fong, 2003. Volumetric analysis predicts hepatic dysfunction in patients undergoing major liver resection. Journal of Gastrointestinal Surgery, 7: 325-30.
7. Kubota, K., M. Makuuchi, K. Kusaka, T. Kobayashi, K. Miki, K. Hasegawa, Y. Harihara and T. Takayama, 1997. Measurement of liver volume and hepatic functional reserve as a guide to decision-making in resectional surgery for hepatic tumors. Hepatology, 26: 1176-1181.
8. Dahm, F., P. Georgiev and P.A. Clavien, 2005. Small for size syndrome after partial liver transplantation: definition mechanisms of disease and clinical implication. American Journal of Transplantation, 5: 2605-2610.
9. Higgins, G.M. and R.M. Anderson, 1931 Experimental pathology of the liver: Restoration of the liver of the white rat following partial surgical removal. Archives of Pathology, 9: 188.
10. Foster, R., M. Zimmerman and J.F. Trotter, 2007. Expanding the donor options: marginal, living and split donors. Clinics in Liver Disease, 11: 417-429.
11. Dancygier, H., 2010. Hepatic regeneration. Clinical Hepatology, 10: 158.
12. Ren, Y.N., N.S. Qian, Y. Tang, Y.H. Liao, W.H. Liu, V. Raut, K.S. Tao and K.F. Dou, 2012. Beneficial effects of splenectomy on liver regeneration in a rat model of massive hepatectomy. Hepatobiliary and Pancreatic Diseases International, 11: 60-65.
13. Samimi, F., W.D. Irish, B. Eghtesad, A.J. Demetris, T.E. Starzl and J.J. Fung, 1998 Role of splenectomy in human liver transplantation under modern-day immunosuppression. Digestive Diseases Sciences, 43: 1931-1937.
14. Neumann, U.P., J.M. Langrehr, U. Kaisers, M. Lang, V. Schmitz and P. Neuhaus, 2002. Simultaneous splenectomy increases risk for opportunistic pneumonia in patients after liver transplantation. Transplant International, 15: 226-232.
15. Nasr, M.M., A.M. Hassan, S.B. Elsebaie, M.A. Elsebae and M.M. Nosseir, 2011. Effect of splenectomy on liver regeneration and function following partial hepatectomy: experimental study. Journal of the Egyptian Society of Parasitology, 41: 601-610.
16. Castaing, D., J. Elcheroth and H. Bismuth, 1999. Conséquences des hépatectomies. Encyclopédie Médico-Chirurgicale, Hépatologie, 7: 5.

17. Magdy Elsebae, M.A. and B. Nadia Abu-Zekri, 2009. Effect of splenectomy on remaining liver function after hepatic resection with inflow-outflow occlusion: Experimental study. World Journal of Medical Sciences, 4(1): 54-60.
18. Fausto, N., 2000. Liver regeneration. Journal of Hepatology, 32: 19-31.
19. Michalopoulos, G.K. and M. DeFrances, 2005. Liver regeneration. Advances in Biochemical Engineering / Biotechnology, 93: 101-134.