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Original Article

PERIOPERATIVE INTERVENTIONS ENHANCES SURVIVAL AND LIVER REGENERATION FOLLOWING EXTENSIVE PORTAL BRANCH LIGATION OF RABBIT LIVER

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Aim: *The purpose of this study was to evaluate the effect of pharmacologic interventions in preventing liver injury, enhancing liver regeneration and prolonging survival following portal vein ligation to 80% of the liver parenchyma.*
Methods: *Rabbits underwent ligation of the portal vein branches to 80% liver parenchyma. One group of animals served as a control. The other group received pharmacologic interventions. Interventions consisted of postoperative oral 20% glucose supplementation to prevent postoperative hypoglycemia, perioperative low-molecular-weight heparin to prevent intrasinusoidal thrombosis and oral antibiotic to prevent bacterial translocation with their injurious effect on liver remnant and hepatocyte proliferation. Outcome measurements included serum liver function tests, glucose levels, and histological assessment of hepatocyte necrosis, apoptosis, mitosis and intrasinusoidal fibrin deposition and length of postoperative survival.*
Results: *Interventions improved survival in the treated group (1.07±0.07 days versus 0.71±0.1 days). Hepatocyte necrosis (31.1±0.02 versus 51.5±0.02), apoptosis (30.4±0.02 versus 40.7 ±0.03) and intrasinusoidal fibrin deposition (5.6±0.8 versus 8.7±1.0) were significantly decreased and mitotic figures (29.1±2.7 versus 15.7±1.2) significantly increased in the treated group compared to control group. Biochemical markers of hepatocyte injury were not different among groups.*
Conclusion: *Pharmacologic interventions improve survival and histological evidence of remnant liver regeneration in animals subjected to portal branch ligation to 80% of rabbit liver.*

Keywords: *Liver regeneration. Liver failure. Bacterial translocation.*

INTRODUCTION

Hepatocellular carcinoma (HCC) is now a common malignancy in Egypt.[1] Surgical resection of HCC remains the only potentially curative treatment and is the gold standard against which all other therapies are compared.[2,3] Unfortunately, most HCC are unresectable at the time of presentation because the small liver remnant following such extensive hepatectomy could not carry out enough function to sustain life.[1,3] Moreover, in Egypt more than 80% of HCC arises in cirrhotic livers with limited functional reserve. The hampered liver functions usually results in postoperative liver failure, making it the leading cause of death following liver resection for HCC.[1,3]

To overcome the problem of postoperative liver failure after extensive resection, portal vein embolization (PVE) clinically and portal vein ligation (PVL) experimentally have been developed.[4-7] The embolized or ligated part becomes atrophic, whereas a nonembolized part, i.e. the future liver remnant, undergoes compensatory hypertrophy before operation.[5,8,9] This procedure has improved the prognosis of patients with HCC after liver resection even in the presence of severe liver cirrhosis and thereby has extended the surgical indications.[9,7,10-12]

The normal liver has substantial reserve capacity and regenerative ability and can safely tolerate a 65% to 75% resection with uneventful recovery.[3,13] After 90% hepatectomy, lethal acute liver failure occurs in 95% of untreated animals.[13,14] Death is not simply due to a loss of

hepatocytes but is the result of ongoing damage to the liver remnant and consequently failure of regeneration.[13] The minimal amount of liver parenchyma that is sufficient to carry out immediate life-sustaining functions has not been yet defined.

Investigators reported enhanced survival with the use of interventions to prevent detrimental factors from damaging liver remnant following extensive hepatectomy. Interventions included postoperative glucose supplementation to prevent postoperative hypoglycemia,[15-17] selective bowel decontamination to prevent bacterial translocation,[18-20] and systemic heparinization to prevent thrombosis of the remnant liver blood vessels.[21,22] To our knowledge, the study of the combined effects of such interventions in the same animal model of extensive hepatectomy or portal vein ligation (PVL) has not been performed.

The goal of this study was to improve survival by preventing remnant liver damage following PVL to 80% of rabbit liver using combined interventions directed at different detrimental factors.

PATIENTS AND METHODS

Animals: Two groups (7 each) of New Zealand White rabbits (2-3 kg) were used. The animals were kept for at least 3 days before use and were fed on commercial food and drinking water ad libitum. The animals were fasted (food) for 24 hours before the procedure. The animals received humane care in compliance with the National Institutes of Health (NIH) guidelines for the use of experimental animals.[23] The study protocol was fully approved by the Faculty Board.

Surgical Procedure: The surgical procedure includes the ligation of portal vein branches to all lobes except the right caudate lobe, which represent 20% of liver parenchyma. The site of ligation of the portal vein of the rabbit liver is shown in Fig. 1.[6] Animals were anesthetized by intramuscular injection into the right thigh of ketamine HCl (5ml-6ml) (50-60 mg/kg) and xylazine HCl (Xyla-Ject, ADWIA, Egypt) (10 mg/kg); spontaneous breathing. Animals received atropine sulfate (0.25 mg/kg) subcutaneously before administration of anesthesia to decrease salivation. All animals received intramuscular administration of antibiotic (penicillin G sodium 50,000 IU + procaine penicillin 150,000 IU + streptomycin sulphate 250 mg, Neobiotic) every day for 3 days.

Experimental Design: All animals were subjected to 80% PVL. Group 1 served as control. Group 2 received low-molecular-weight (LMW) heparin (enoxaparin sodium, Clexan) 0.5 mg/kg/day SC started two hours before PVL, oral metronidazole 125 mg twice daily started 1 day before PVL, and 20% glucose ad libitum postoperatively. Postoperatively, animals were observed for the development of signs of impending death (irregular breathing, sluggish reflexes to painful stimuli). At that time, laparotomy was performed, liver specimens were taken, and animals were sacrificed with exsanguination.

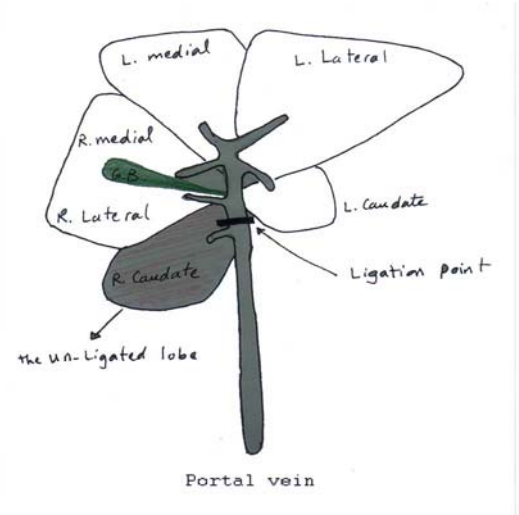


Fig 1. Anatomical diagram of the rabbit liver and the point of ligation of portal vein feeding 80% of liver.

Biochemical Analysis: Blood samples were withdrawn before and 24 hours after PVL and serum was separated and analyzed with automated analyzer for alanine transaminase (ALT), γ glutamyl transferase (GGT), total bilirubin, prothrombin time (PT), and glucose using commercial kits.

Histological Examination: Liver specimens obtained at time of sacrifice of both ligated and non-ligated lobes of the liver were fixed in 10% formalin. Sections (5 um thick) of the specimens were cut and stained with hematoxylin and eosin (HE).[4] Four or five livers were analyzed for each experimental group. Morphometric analysis of light microscope images of the hepatic lobules, at a magnification of ×400, was performed using Leica Q500 MCO analyzer (Wetzlar, Germany). The histologist was not blinded regarding the group assignment, as the morphometric analysis was performed by an automated analyzer. Morphometric analysis included the following:

1. Extent of hepatic injury was estimated by documenting the number of hepatocytes exhibiting evidence of necrosis or apoptosis. Evidence of necrosis includes homogenous eosinophilic cytoplasm and faintly stained nuclei (karyorrhexis and karyolysis).The presence of apoptotic hepatocytes was examined using standard morphological criteria: shrunken hepatocytes and single-rounded cells or fragments showing aggregation of chromatin into

uniform dense masses and irregular nuclear membranes were considered apoptotic.[4] The number of apoptotic hepatocytes was expressed as percent of the total number of hepatocytes examined.

2. Extent of regeneration was determined by calculating hepatocytes with mitotic figures in 10 visual fields selected at random.

3. Intrasinusoidal fibrin deposition was documented using phosphotungstic acid-hematoxylin (PTAH) staining.[22-26] The number of sinusoids exhibiting intrasinusoidal fibrin per high power field was counted.

Statistical Analysis: The results are expressed as the mean ± the standard error (SE). Statistical analysis was performed with the software Statistical Package for Social Sciences, SPSS version 8 (SPSS, Chicago, IL). A p value of less than 0.05 was considered statistically significant.

RESULTS

Changes in postoperative glucose and liver function tests: Serum ALT, GGT, PT, total bilirubin, and glucose concentrations before and after PVL of the groups are summarized in Table 1. Pre-intervention values were similar among groups and were within the reference range. Postoperative values differed significantly from preoperative values, but there were no significant differences in the postoperative liver function values among groups. Postoperative glucose concentrations were significantly lower in the control group, but not different in the treated group compared with preoperative values.

Histopathologic findings: In all groups, the non-ligated caudate lobe examined at sacrifice revealed a gross appearance of pink color with tense enlargement, whereas the ligated lobes were shrunken and pale (Fig. 2). Microscopic findings in the ligated and non-ligated lobes are depicted in figures 3, 4 and 5. Ligated lobes exhibited massive necrosis (Fig. 3). The non-ligated caudate lobes of the control group exhibited moderate degree of hepatocyte injury (vacuolated cytoplasm of most of hepatocytes accompanied with different stages of nuclear degeneration), dilated sinusoids, and inflammatory cell infiltration (Fig. 4a,b). The non-ligated caudate lobes of group 2 (Fig. 5) showed mild degree of hepatocyte injury (less abundant cytoplasmic vacoulation and nuclear degeneration).

Morphometric analysis: The results of morphometric analysis are summarized in Table 2. The percent of hepatocytes showing nuclear changes denoting necrosis or apoptosis and fibrin deposition in the sinusoids of the non-ligated lobes were significantly lower in group 2 compared with the control group. The mitotic figures in the non-ligated lobes were significantly higher in group 2 compared with the control group.

Survival Analysis: Survival rate in group 2 was significantly better than in group 1 (1.07±0.07 days and 0.71±0.1 days, respectively).

Table 1. Summary of serum glucose and liver function tests results after PVL.

Test (units)	Pre-study	Group 1	Group 2
ALT (IU/L)	35 ± 3	1288 ± 320†	1163 ± 251†
GGT (IU/L)	17 ± 1.4	65 ± 10.2†	67 ± 9.5†
PT (seconds)	12.5 ± 0.03	12.7 ± 0.06†	12.8 ± 0.1†
Bilirubin (mg/dl)	0.5 ± 0.02	5.3 ± 0.5†	5.2 ± 0.5†
Glucose (mg/dl)	263 ± 18	90 ± 53†	239 ± 44

† $p < 0.05$ compared with preoperative values in the same group.

Table 2. Summary of morphometric analysis.

Parameters	Group 1	Group 2
Necrosis	51.5 ± 0.02	31.1 ± 0.02†
Apoptosis	40.7 ± 0.03	30.4 ± 0.02†
Mitotic index	15.7 ± 1.2	29.1 ± 2.7†
Intrasinusoidal fibrin	8.7 ± 1.0	5.6 ± 0.8†

†statistically significant difference compared with control group.

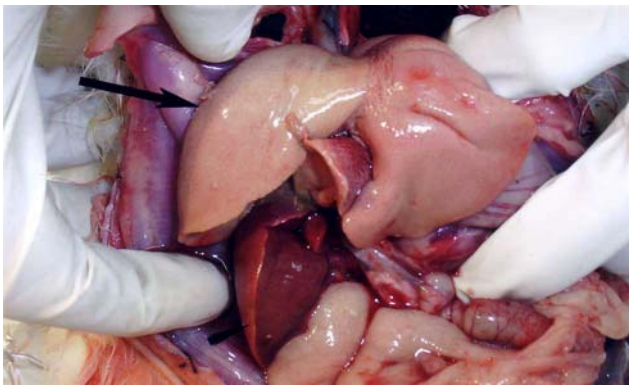


Fig 2. Portal vein ligation to 80% resulted in blanching of liver lobes of ligated portal vein (arrow) while the non-ligated caudate lobe (arrowhead) retains its pink color.

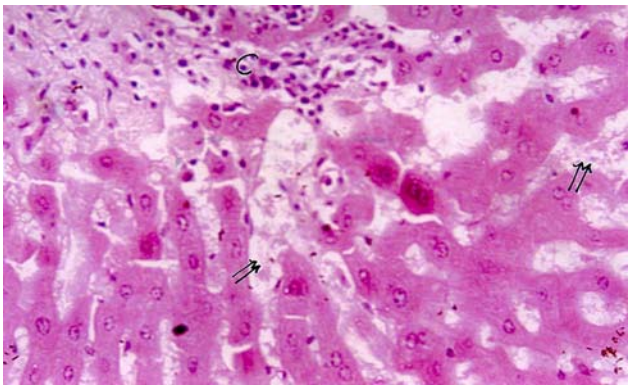
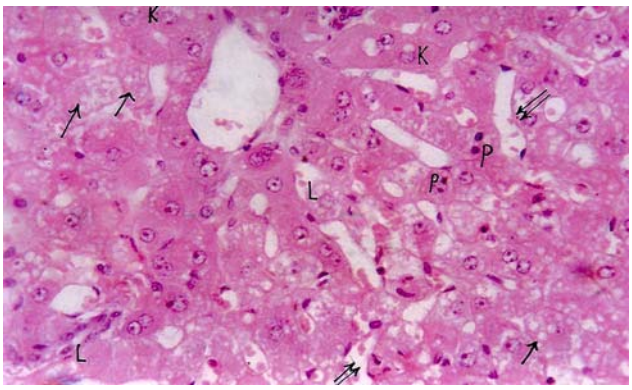


Fig 3. Photomicrograph of a ligated liver lobe showing homogenous eosinophilic cytoplasm of the hepatocytes (mucoid degeneration), dilated congested sinusoids (double arrows), and cellular infiltration (C). (HE × 400).



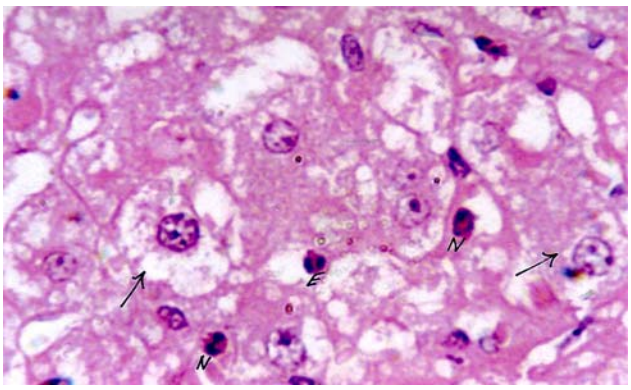


Fig 4. Left, photomicrograph of a non-ligated lobe of group 1 showing vacuolated cytoplasm of most hepatocytes (arrow). Some hepatocytes show different stages of nuclear degeneration such as pyknosis (P), karyorrhexis (K), and karyolysis (L). Dilated sinusoids are also shown (double arrows). (HE × 400). Right, a higher magnification showing vacuolated hepatocytes (arrow), eosinophils (E) and neutrophils (N). (HE × 1000).

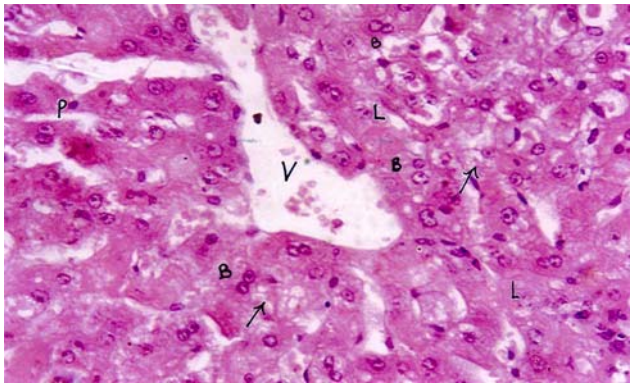


Fig 5. Photomicrograph of a non-ligated lobe of group 2 showing dilated central vein (V). Many hepatocytes are seen to be binucleated (B). Few hepatocytes show vacuolated cytoplasm (arrow) or nuclear pyknosis (P) and

karyolysis (L). (HE × 400).

DISCUSSION

In this study, pharmacologic interventions significantly improved survival rate of the treated animals compared with those of the control group. This is supported by histopathologic analysis, which demonstrated significant decrease in hepatocyte injury and apoptosis and increase in mitotic figures as well as diminished intrasinusoidal fibrin deposition. The results of liver function tests, however, were not significantly better in the treated groups compared with control group. Urayama et al in a similar study of 80% PVL of rabbit liver found that the compensatory hypertrophying liver is enlarging without functional augmentation in the early period after PVL and that the hepatic functional reserve of the unligated lobe after resection of the ligated lobe was suppressed to a significantly low level during the first 7 days.[6]

Postoperative hypoglycemia is one of the most serious problems after massive hepatic resection.[27] After 70% hepatectomy, the remaining liver extracts less insulin per gram. This contributes to the ability of the remnant liver to continue to produce glucose and maintain euglycemia after major liver resection.[28] However, glucose intolerance developed in the early critical phase after massive hepatic resection in patients and animals. Sarac et al found that preoperative fasting and postoperative 20% glucose consumption improves survival after 90% hepatectomy in rats. They concluded that fasting before hepatectomy shifts energy utilization to fat oxidation and gluconeogenesis, which appears to ameliorate liver failure after hepatectomy in this severe model of hepatic resection.[16] Many investigators agree that alteration of the energy substrate from glucose to free fatty acid occurs during the early stage after partial hepatectomy.[29,30]

Recent molecular investigations shed some light on the genetic mechanisms behind hypoglycemia that follows liver injury. In a study of the hepatic gene expression following lipopolysacchride administration in dogs using microarray analysis, Higgins et al found decreased plasma levels of glucose and that genes involved with glucose regulation were downregulated.[31]

In our study, preoperative fasting for 12 hours and postoperative oral 20% glucose supplementation in group 2 resulted in increase in postoperative blood glucose levels compared with those of the control group and were not different from preoperative values. Thus, correcting hypoglycemia have contributed to the significantly improved survival rate in group 2 compared to the control group.

There is growing body of evidence that bacterial translocation to the liver or endotoxemia often takes place in the early time period after major hepatectomy and thus the induced hepatic inflammation promotes hepatic failure.[7,32] Moreover, it has been also found that TNF-α released from endotoxin-activated hepatic macrophages induces morphological and functional alterations to sinusoidal endothelial cells, which leads to microcirculatory disturbance and ultimately to hepatic necrosis, suggesting the involvement of this cytokine in the development of postoperative hepatic failure.[7]

Arai et al reported that selective bowel decontamination significantly reduced endotoxin concentration in portal blood and attenuated serum ALT activity, TNF-α concentration, and histological extent of liver necrosis after orthotopic liver transplantation in rats.[18] Kakkos et al also found that nonabsorbable antibiotics reduce bacterial and endotoxin translocation in hepatectomized rats.[33]

Our results indicate that administration of nonabsorbable antibiotics significantly ameliorated hepatocyte necrosis and apoptosis and increased mitotic figures.

It has been shown that the microcirculatory disturbance caused by microthrombus formation and sinusoidal endothelial cellular injury is one of the causes of posthepatectomy liver dysfunction.[26] Mochida et al found that fibrin deposition developing in 70% hepatectomized rats after endotoxin administration may be caused by deranged blood coagulation in the hepatic sinusoids through increasing tissue factor activity, an initiator of blood coagulation, in Kupffer cells and minimal tissue factor pathway inhibitor (TFPI) and thrombomodulin in endothelial cells.[22] Arai et al also reported that Kupffer cells are activated to increase tissue factor activity in rats following 70% liver resection and this activation was significantly attenuated by oral administration of nonabsorbable antibiotics suggesting that substances derived from bacteria in the gut, such as endotoxin, may enter the portal blood and activate Kupffer cells causing liver injury.[18] This data was supported by several studies that showed that anticoagulant factors such as thrombomodulin, activated protein C and TFPI administration increased anticoagulant activity on the hepatic sinusoidal wall and inhibited intrasinusoidal fibrin deposition and posthepatectomy liver dysfunction.[24,26,34]

Additionally, systemic heparinization has been shown to

exert a protective effect on hepatic injury in pigs and rabbit models of hepatic ischemia/reperfusion injury.[21,35] Moreover, heparin injections significantly augmented liver regeneration after portal branch ligation in both normal and cirrhotic rats and improved the survival rate after extensive hepatectomy in the cirrhotic rats.[36]

Recently, the genetic bases of coagulation disorders following liver injury and hepatectomy are being discovered. Xu et al using cDNA microarray technology to identify the genes differentially expressed in the regenerating rat liver after successive partial hepatectomy found that the gene encoding coagulation factor 2 protease inhibitor was downregulated early after hepatectomy to facilitate blood circulation for liver regeneration but at 12-h time point was upregulated to hamper great loss of blood by hepatectomy.[37]

In this study, we demonstrated that administration of LMW heparin significantly decreased intrasinusoidal fibrin deposition. Decreased fibrin deposition in the non-ligated caudate lobe of liver has contributed to improved survival in the treated group.

In conclusion, this study demonstrated that pharmacologic interventions in the form of postoperative glucose supplementation, perioperative anticoagulation with LMW heparin, and nonabsorbable antibiotics significantly improved survival rate, decreased hepatocyte injury and increased hepatocyte mitosis following PVL to 80% of the liver. These interventions, however, are not sufficient to prolong survival enough for liver regeneration until its completion. The goal of future experimental studies should aim to support liver regeneration in the future liver remnant using other therapeutic approaches such as gene therapy in order to increase liver parenchymal reserve before embarking on extensive (>70%) resection or embolization.

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