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# Effect of Splenectomy on Remaining Liver Function after Hepatic Resection with Inflow-Outflow Occlusion: Experimental Study

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**Abstract:** Experimentally, inflow-outflow occlusion of the liver by total vascular exclusion (TVE) causes more prominent ischemia/reperfusion injury compared with inflow occlusion only by Pringle maneuver. This study investigated the effect of prior splenectomy on the remnant liver function following a two-thirds partial hepatectomy with TVE in hamsters. Thirty hamsters were assigned to 3 main groups (10 in each group). Animals in group A were subjected to a laparotomy only (sham operation). In group B, animals were subjected to a two-thirds partial hepatectomy with total vascular exclusion for 30 min. Animals in group C were subjected to the same procedure, but with a prior splenectomy just before hepatectomy. Blood was sampled for the measurement of routine liver function parameters (serum total bilirubin, alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT)) as well as plasma lecithin cholesterol acyl transferase (LCAT) enzymatic activity which was found a valuable and sensitive indicator for early detection of hepatic damage and dysfunction (by an enzymatic colorimetric method) and serum hyaluronic acid (HA) which is specifically significant for assessment of the early microvascular liver damage (by a radiometric assay) at the following time points: at laparotomy, 24 and 72 hours after surgery. It was found that in group A, the mean values of serum total bilirubin, ALT, GGT and HA measured at 24 and 72h after the surgical procedure were not significantly altered from corresponding mean values measured at laparotomy, while in groups B and C mean values of serum total bilirubin, ALT, GGT and HA were significantly elevated and plasma LCAT significantly decreased versus corresponding laparotomy mean values at both time points (24 and 72h posthepatectomy). Mean values of the aforementioned parameters were not significantly different between groups B and C at the 24 h posthepatectomy assessment. However, at 72h posthepatectomy, group C i.e. the group subjected to prior splenectomy just before hepatectomy showed significantly lower mean values of serum total bilirubin, ALT, GGT and HA and significantly higher mean value of plasma LCAT as compared to group B. It was concluded that prior splenectomy ameliorated the hepatic ischemia / reperfusion injury with improvement of the remnant liver function after partial hepatectomy with total hepatic vascular exclusion in hamsters.

**Key words:** Hepatic ischemia/reperfusion injury · Total hepatic vascular exclusion · Splenectomy

## INTRODUCTION

Temporary occlusion of the hepatoduodenal ligament (Pringle maneuver) or total vascular exclusion (TVE) , which is often used at liver resection to reduce blood loss, was found to induce hepatic ischemia/reperfusion (I/R) injury with harmful effect on the metabolic function of hepatocytes [1,2,3]. Total hepatic vascular exclusion (TVE) involving portal triad exclusion and clamping of the inferior vena cava (IVC) below and above the liver can reduce blood loss during transection of the hepatic parenchyma. However, inflow-outflow occlusion of the liver by TVE in the rat model causes more prominent ischemia/reperfusion injury in the form of oxidative stress compared with inflow occlusion only by Pringle maneuver [1,4].

Splenectomy or splenic artery ligation was reported to ameliorate the experimentally induced

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hepatic ischemia and reperfusion injury after partial hepatectomy using Pringle maneuver with improvement of the remnant liver function [5-7]. By Medline search, no experimental study was found designating the effect of splenectomy on ischemia/reperfusion injury and the remnant liver function after partial hepatectomy under total vascular exclusion.

Liver function tests (LFTs) are measurements of blood components that simply provide a lead to the existence, the extent and the type of liver damage. Serial use of LFTs is of most value in following the progress or resolution of liver disease [8].

Liver damage caused by I/R occurs at the microvascular as well as the parenchymal level [9-11]. In particular, microvascular damage involves the sinusoidal endothelial cells, whereas parenchymal damage affects mainly the hepatocytes [12,13]. Several studies have shown that injury of the sinusoidal endothelial cells can be assessed by their ability to take up hyaluronic acid (HA). HA is a glycosaminoglycan produced by connective tissue throughout the body and is predominantly taken up and metabolized by endothelial cells in the liver [14,15]. Thus, the use of serum levels of hyaluronic acid (HA) is specifically significant for assessment of the early microvascular liver damage [16, 17]. Parenchymal damage was assessed by the release of conventional liver enzymes into the blood [17]. The serum aminotransferase activities are a measure of the integrity of liver cells. Thus, raised activities of the aminotransferases (AST and ALT) indicate hepatocellular damage. In acute hepatic damage, changes in gamma-glutamyl transferase (GGT), a microsomal liver enzyme, activity parallel those of the aminotransferases. Increased bilirubin concentration and increased alkaline phosphatase activity indicate the presence of cholestasis, a blockage in bile flow [8]. Lecithin cholesterol acyl transferase (LCAT) enzyme (a serine type esterase) is a glycoprotein responsible for the esterification of cholesterol in plasma. It is synthesized in the liver, released into the circulation, where it produces its action and is primarily activated by apo A-I [18]. The assessment of LCAT was found a valuable and sensitive indicator for early detection of hepatic damage and dysfunction [19,20].

The current study investigated the effect of prior splenectomy on the remnant liver function following partial hepatectomy with total vascular exclusion through quantitation of serum HA levels, plasma LCAT activity in addition to conventional liver function parameters: serum total bilirubin, alanine aminotransferase (ALT) and γ glutamyl transferase (GGT) enzymatic activities at laparotomy, 24 and 72 hours following the surgical procedure.

## MATERIALS AND METHODS

**Experimental Design:** All experiments were conducted according to the Guide for the Care and Use of Laboratory Animals of Theodore Bilharz Research Institute. All animals were housed under standard environmental conditions and allowed tap water and rat pellets ad libitum throughout the study.

Thirty hamsters (Golden hamsters, Mesocricetus Auratus) weighing 100-120 g were assigned to 3 main groups (10 in each group). Animals in group A were subjected to a laparotomy, then the abdomen was closed (sham operation). In group B, animals were subjected to a two-thirds partial hepatectomy with total vascular exclusion for 30 min. In group C, animals were subjected to the latter procedure with a prior splenectomy just before hepatectomy. Blood sampling for the measurement of serum total bilirubin, alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), serum hyaluronic acid (HA) and plasma lecithin cholesterol acyl transferase (LCAT) was performed at laparotomy, 24 and 72 hours after the surgical procedure in all groups.

**Surgical Technique:** All animals were fasted for 12 hours before operations. The hamsters were generally anaesthetized with ether after which the abdomen was shaved and swabbed with iodine in preparation for surgery. The surgical procedures were performed under aseptic, but not sterile conditions. The same researcher performed all surgical procedures. After midline laparotomy was performed, in the sub-xiphoid area, the abdomen was opened and the liver was mobilized. A 2-0 Vicryl thread was passed to surround the liver hilum and another thread was passed to surround the hepatic veins. Those threads were tied but without a knot. Because the hamster's liver is lobulated, the median and lateral lobes were then removed *en bloc* and then the threads were released after 30 minutes. The abdomen was closed with a running, single-layer Vicryl 5/0 suture.

**Liver Injury Markers Assessment:** Total serum bilirubin, ALT and GGT were measured in fresh blood sera within 36h of collection (standard methods).

Hyaluronic acid (HA) was measured in blood serum, stored at-70°C until assayed, by a radiometric assay kit (Kabi Pharmacia, Uppsala, Sweden) according to the method of Tengblad [21].

Lecithin cholesterol acyl transferase (LCAT) activity was determined in plasma, separated from heparinized blood and stored at -70°C until assessed, by an enzymatic colorimetric method according to Nagasaki and Akanuma [22]. Synthetic dipalmitoyl lecithin solution was added to the incubation medium (at 37°C) to improve the linearity of the LCAT reaction. The change in the free cholesterol content was measured after incubation by combined enzymatic method using cholesterol oxidase and peroxidase in the presence of phenol and 4-aminoantipyrine. Absorbance values were read photometrically at 500 nm and LCAT activity was determined by the difference in absorbances taken at 0 and at 2 hours. Measurements were performed at laparotomy, 24 and 72 hours after the surgical procedure in all groups.

**Statistical Methodology:** Results of assessed parameters were expressed as mean values ± SD. The paired Student *"t"* test was used to analyze the response of assessed variables to the surgical procedure in each group. The differences between studied groups at the same time point were analyzed by the unpaired Student *"t"* test. Values of P less than 0.05 were regarded as significant. Data were plotted by line chart (Microsoft Excel, 2003 Worksheet).

## RESULTS

Results were expressed as mean values ± SD and depicted in Table 1 and Figures (1-4). All animals in group A (sham operation) survived. In partially hepatectomized hamsters with 30 min of total vascular exclusion (Group B), seven out of 10 died within one week, while only one out of 10 died when splenectomy was performed prior to hepatectomy (Group C). Survival rates after the same period of ischemia were statistically different between groups B and C (30% and 90% respectively, P=0.02). As to liver function parameters: in group A, mean values of serum total bilirubin, ALT, GGT, HA and plasma LCAT measured at 24h and 72h after the surgical procedure exhibited insignificant differences from those measured at laparotomy. Meanwhile, mean values of serum total bilirubin, ALT, GGT and HA determined at 24h and 72 h posthepatectomy were found to be significantly elevated, whereas plasma LCAT activity significantly reduced in groups B and C as compared to corresponding laparotomy mean values as follows: in group B (P<0.0001 for total bilirubin, ALT, GGT and HA; P=0.0002 for LCAT) at both time points versus their corresponding laparotomy mean values; and in group C (P<0.0001 for total bilirubin,ALT, GGT and HA, P=0.0004 for LCAT) at 24 h and (P=0.0004 for total bilirubin, GGT, HA and LCAT, P<0.0001 for ALT) at 72 h versus their corresponding laparotomy mean values.

Concerning group differences, it was found that at the 24h posthepatectomy assessment, mean values of the aforementioned laboratory parameters were not significantly different between groups B and C, whereas at 72h posthepatectomy mean values of total

Table 1: Changes in routine liver function tests, serum HA and plasma LCAT

| Parameters | Group | At Laparotomy | 24h after hepatectomy | 72h after hepatectomy |
|---|---|---|---|---|
| ALT U/L | A | 16±0.9 | 17±1.9 | 18±0.4 |
| | B | 19±2.9 | 108.3±6.5••• | 106±2.3••• |
| | C | 15±5.0 | 103.7±2.1••• | 72±0.6••• *** |
| GGT U/L | A | 55±0.1 | 54±2.3 | 54±0.3 |
| | B | 54±3.1 | 206.5±7.2••• | 176±0.25••• |
| | C | 57±2.1 | 209±1.5••• | 98±0.13• *** |
| T-Bilirubin mg/dL | A | 0.11±0.1 | 0.14±1.3 | 0.13±0.3 |
| | B | 0.14±0.13 | 4.5±0.25••• | 5.5±0.75••• |
| | C | 0.21±0.12 | 2.2±0.09••• | 1.2±0.9• *** |
| HA(ng/ml) | A | 61±3.0 | 51±0.4 | 53±0.1 |
| | B | 63±5.0 | 205±2.58••• | 231±1.2••• |
| | C | 51±4.4 | 191±1.15••• | 101±0.03• * |
| LCAT nmol/ml/hr | A | 47.2±0.1 | 42.5±0.5 | 48.9±2.1 |
| | B | 46.3±0.2 | 32.4±2.0•• | 31.0±0.1•• |
| | C | 44.9±0.5 | 29.1±2.1• | 41.6±2.3• ** |

•••= significant at P<0.0001, •• = significant at P=0.0002, • = significant at P = 0.0004 versus value at laparotomy, *** = significant at P value less than 0.0001, ** significant at P value <0.0007, * = significant at P value = 0.006 in group C versus group B at 72h assessment after hepatectomy

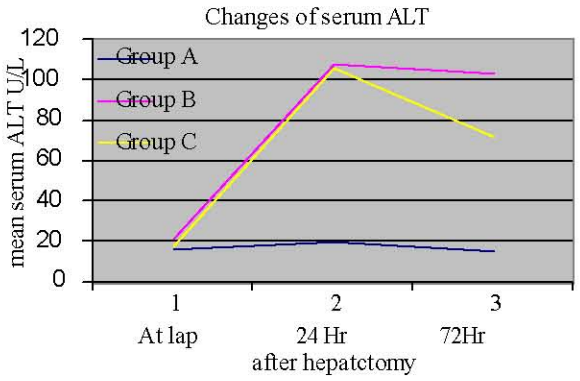


Fig. 1: Serum ALT (U/L) before and after partial hepatectomy with TVE

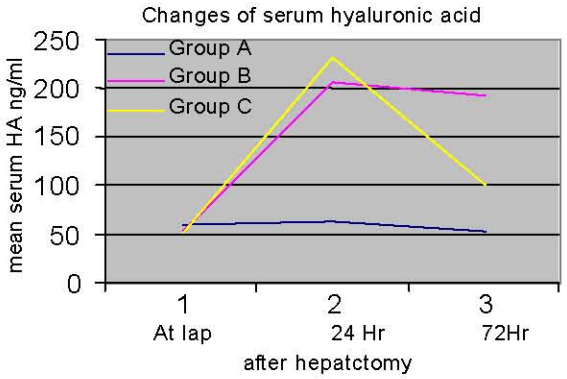


Fig. 3: Serum HA (ng/ml) before and after partial hepatectomy with TVE

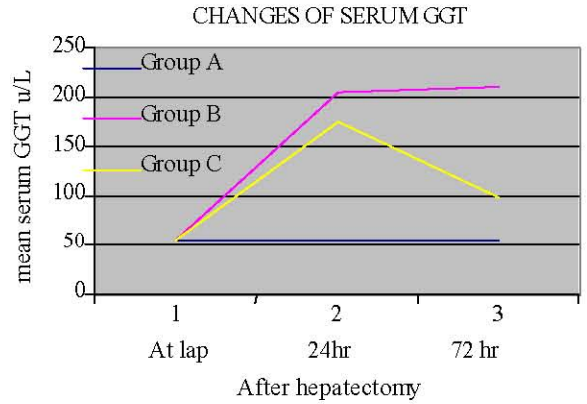


Fig. 2: Serum GGT (U/L) before and after partial hepatectomy with TVE

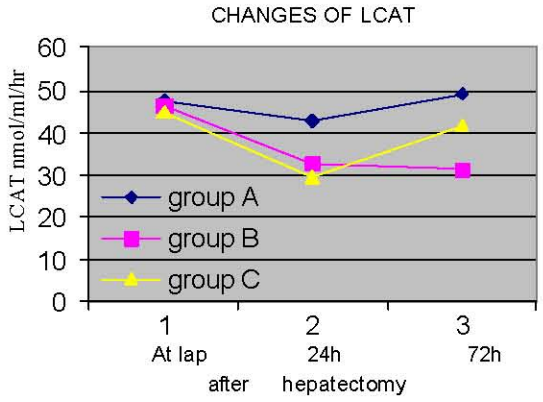


Fig. 4: Plasma LCAT (nmol/ml/hr) before and after partial hepatectomy with TV

bilirubin, ALT, GGT and HA were significantly lower in group C as compared to group B ($P<0.0001$ for total bilirubin, ALT and GGT, $P=0.0006$ for HA) and mean value of LCAT significantly higher in group C versus B ($P<0.0007$) (Table 1), Fig. (1-4).

## DISCUSSION

Intraoperative bleeding is a main concern during liver resections and mortality and morbidity are clearly correlated with the amount of blood loss. However, the method of vascular occlusion employed to reduce that blood loss is controversial. Performing the Pringle maneuver (inflow occlusion only), that has been used widely during liver transection, resulted in less blood loss and better preservation of liver function in the early postoperative period [3,23]. Total hepatic vascular exclusion (TVE) involving portal triad exclusion and clamping of the inferior vena cava (IVC) below and above the liver can reduce blood loss during transection of the hepatic parenchyma. However, it will produce ischemic injury to the remaining liver greater than during resection with portal pedicle clamping. Moreover, the degree of ischemic injury to the hepatocytes may be accentuated in the presence of underlying liver disease [24-27].

Several strategies have been used to minimize ischemic injury during liver surgery, especially in patients with abnormal liver parenchyma [28,29]. Prior splenectomy in addition to partial hepatectomy with Pringle maneuver has been designed experimentally as one of those strategies to ameliorate ischemia/ reperfusion (I/R) injury in the remnant liver in rat model [5]. The beneficial effect of splenectomy when performed with partial hepatectomy was explained to be due to enhancement of the regenerative capacity of the liver by removal of inhibitory factor released from the spleen [30].

As to liverfunction parameters assessed in the current study, statistical analysis revealed that in group A, mean values of serum total bilirubin, ALT, GGT, HA and plasma LCAT measured at 24h and 72h after the

surgical procedure exhibited insignificant differences from those measured at laparotomy. Meanwhile, mean values of serum total bilirubin, ALT, GGT and HA determined at 24 h and 72 h posthepatectomy were found to be significantly elevated, whereas plasma LCAT activity significantly reduced in groups B and C as compared to corresponding laparotomy mean values as follows: in group B ($P<0.0001$ for total bilirubin, ALT, GGT and HA; $P=0.0002$ for LCAT) at both time points versus their corresponding laparotomy mean values; and in group C ($P<0.0001$ for total bilirubin, ALT, GGT and HA, $P=0.0004$ for LCAT) at 24 h and ($P=0.0004$ for total bilirubin, GGT, HA and LCAT, $P<0.0001$ for ALT) at 72 h versus their corresponding laparotomy mean values.

Concerning group differences, it was found that at the 24h posthepatectomy assessment, mean values of the aforementioned laboratory parameters were not significantly different between groups B and C, whereas at 72h posthepatectomy mean values of total bilirubin, ALT, GGT and HA were significantly lower in group C as compared to group B ($P<0.0001$ for total bilirubin, ALT and GGT, $P=0.0006$ for HA) and mean value of LCAT significantly higher in group C versus B ($P<0.0007$).

Thus, this experimental study confirmed the role of prior splenectomy in preserving the remnant liver functions after a two-thirds partial hepatectomy when performed under total hepatic vascular exclusion (TVE) for 30 minutes as revealed by amelioration of the elevation of serum total bilirubin, ALT, GGT and HA as well as amelioration of the decrease of plasma LCAT in the group of hamsters in which splenectomy preceded hepatectomy (Group C). That amelioration was not found when splenectomy was not performed with hepatectomy (Group B). HA is a glycosaminoglycan produced by connective tissue throughout the body and is predominantly taken up and metabolized by endothelial cells in the liver [14,15]. Thus, the use of serum levels of hyaluronic acid (HA) is specifically significant for assessment of the early microvascular liver damage [16,17]. Elevation of serum HA was reported to occur when its degradation was reduced after reperfusion in unresected liver because of hepatic sinusoidal injury. Then, the release of AST and GGT occurs only after reperfusion of the ischemic remnant liver parenchyma following hepatectomy [17]. Lecithin cholesterol acyl transferase (LCAT) enzyme is responsible for esterification of cholesterol in plasma [18] and was found a valuable and sensitive indicator for early detection of hepatic damage and dysfunction [19,20]. Since it is synthesized by hepatocytes, LCAT may be added to the profile of liver functions as it is a simple, not costly and not laborious test and can be performed as a routine test at any laboratory [19]. Clinical implication of this experimental study is that it confirms the recently reported beneficial clinical effects of splenectomy on the integrity of the residual liver following liver resection for hepatocellular carcinoma in cirrhotic patients with hypersplenism and portal hypertension [31-33].

Taken together, displayed results allowed the current authors to conclude that prior splenectomy can ameliorate the hepatic ischemia/reperfusion injury in the unresected liver with improvement of remnant liver function after a two-thirds partial hepatectomy when performed under total hepatic vascular exclusion for 30 minutes in hamsters as revealed by amelioration of the elevation of serum total bilirubin, ALT, GGT and HA and amelioration of the decrease of plasma LCAT, unlike the case when splenectomy was not performed with partial hepatectomy (Group B). Its implication is confirmation of beneficial clinical effects of splenectomy on residual liver function following liver resection for liver tumors.

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