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RESEARCH ARTICLE

Future Liver Remnant Regeneration Post Hepatectomy: Histologic Changes in an Animal Model

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ABSTRACT:

Background: The liver possesses remarkable regenerative abilities following parenchymal injury. This study aimed to assess and document the microscopic regenerative changes in the rat liver using a partial hepatectomy model.

Methodology: Adult male Sprague Dawley rats weighing approximately 200-300 grams were subjected to partial hepatectomy, leaving approximately 70% future liver remnant. The rats were euthanized at designated time intervals (1/2 hour, 48 hours, 72 hours, 7 days, 11 days, and 14 days) for histological analysis of the liver.

Results: Liver regeneration was observed within 1 week of partial hepatectomy. Cytoplasmic vacuolization increased until day 7, but decreased significantly during the regenerative process. Disruption of cytoplasmic membranes and blurring of cell-to-cell and cell-to-sinusoidal space boundaries decreased notably from day 11. Necrosis involving central veins and portal tracts was observed on the first day after hepatectomy. By day 7, the portal tract exhibited granulomatous inflammation with conglomerates of epithelioid macrophages forming giant cells. The sinusoidal spaces displayed increased lymphocyte volume on day 7. Patchy portal inflammation consisting of lymphocytes, plasma cells, occasional eosinophils, and monocytes was observed from day 7 post-hepatectomy.

Conclusion: In this partial hepatectomy animal model, the inflammatory cascade was evident through confluent necrosis affecting approximately 10% of the hepatic parenchyma, along with subcapsular infarction. Cytoplasmic vacuolization affected approximately 50% of the cells. The study documented various histological changes during the post-hepatectomy period, demonstrating the initiation of hepatocyte regeneration within one week.

Keywords: Liver, regeneration, histology, hepatectomy, remnant.

Introduction:

The liver is a vital organ involved in various metabolic, immune, and detoxification processes.[1,2] It is not unusual for hepatobiliary surgeons to remove portions of the liver parenchyma after traumatic injuries or affliction with malignancies.[3,4] After operative removal, the remaining parenchyma is known as the functional liver remnant (FLR), and it is solely responsible to maintain the vital life processes described above.[5,6] Fortuitously, the FLR possesses a remarkable ability to regenerate after hepatocyte injury.

It is important that researchers fully understand the regenerative processes occurring in the FLR as they are unique to this internal viscus. An understanding of the processes can also help to alter FLR regeneration, thereby reducing post-hepatectomy liver failure[7] and post-hepatectomy mortality,[8] both of which are serious complications after liver resections.

The partial hepatectomy model was first described by Higgins and Anderson in 1931, and it has been instrumental to study the processes occurring during liver regeneration. [9,10,11] Using this animal model, several researchers have explored the effect of physiological and systemic factors on FLR regeneration,[12,13,14] but there has been little focus on the histological changes that occur during FLR regeneration. The aim of this study was to document the histologic changes at each stage of FLR regeneration. This knowledge can be extrapolated to clinical settings and potentially enhance outcomes for patients undergoing liver resections.

Methods:

This study received approval from the Institutional Review Board at the University of the West Indies, Mona Campus. All animal experiments adhered to the guidelines outlined in the Animal Welfare Act and Regulations for Use of Animals in Research. Adult male Sprague Dawley (SD) rats weighing 200-300 grams were used for the experiments.

a. Study Preparation:

In this study, 18 SD rats were anesthetized with intra-peritoneal sodium phenobarbitone at a dose of 30mg/kg. The abdomen was then prepared and incised sharply. Approximately 30% of the SD rat liver was removed by transecting the parenchyma at the left lateral lobe near the inferior vena cava. The abdomen was closed using 3/0 non-resorbable silk sutures.

b. Study Groups:

The SD rats were divided into six groups, each consisting of three animals. Rats in each group were euthanized at different time points: 30 minutes, 48 hours, 72 hours, 7 days, 11 days, and 14 days. Liver biopsies were performed after reopening the abdomen. Tissue samples were processed and sectioned at 10µm using a rotary microtome. Haematoxylin & Eosin or Silver Staining (Gordon and Sweets' Technique) methods were employed for histological staining.

c. Criteria for Assessment:

Liver sections were coded randomly and evaluated by a single observer using the following 12 criteria to assess hepatic abnormalities: (1) morphology of the liver parenchyma; (2) presence of bile duct proliferation; (3) number of polymorphonuclear cells in the portal tracts; (4) spilling of inflammatory cells across the limiting plate; (5) potential inflammation of/or around the central vein; (6) presence of cell dropout or necrotic parenchymal cell; (7) number of lymphocytes observed in the portal tracts; (8) morphology of portal tracts; (9) number of PAS-positive macrophages in the parenchyma; (10) presence of steatosis; (11) morphology of portal tracts; and (12) number of portal tracts and central veins per low power field (100 x magnification).

d. Microscopic Examination:

All slides were examined using a morphometric grid. Similar-sized portal tracts were selected by identifying portal veins with diameters of 20-25 µm. The numbers of bile ducts, lymphocytes, macrophages, etc., were counted in at least 5 portal tracts and 5 randomly selected low-power (100x) fields of the parenchyma using the grid. A scoring system was implemented, assigning values of 0, 1, 2, or 3 for each parameter (Table 1). Once the code was revealed, mean ± SD values were calculated for parameters that could be quantitated from 6-12 sham-operated animals of each rat strain (Table 1).

Table 1: Scoring System for 12 Histological Criteria of Hepatic Regeneration

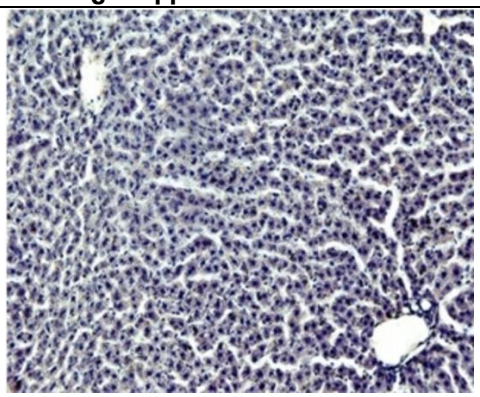
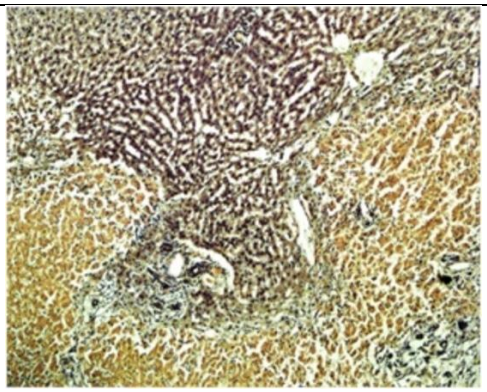
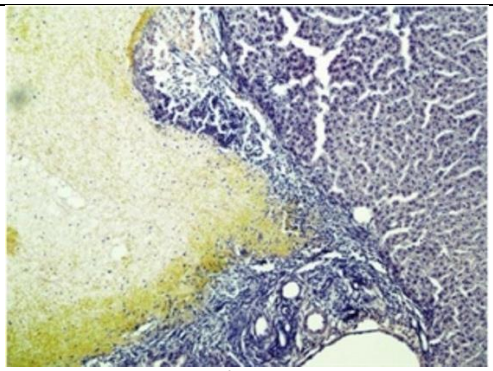
Score	0	1	2	3
Parameter value	> X+1(SD)	> X+1(SD)	> X+2(SD)	> X+3(SD)
Key: X = Mean value observed for each parameter in sham operated rats; SD standard deviation				

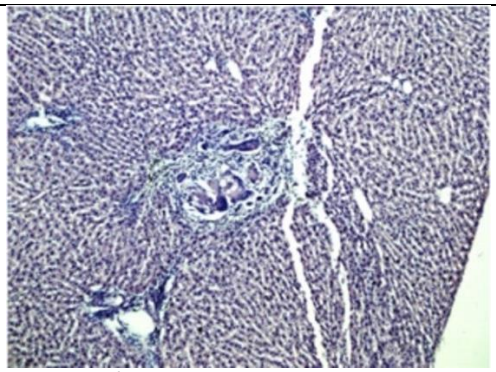
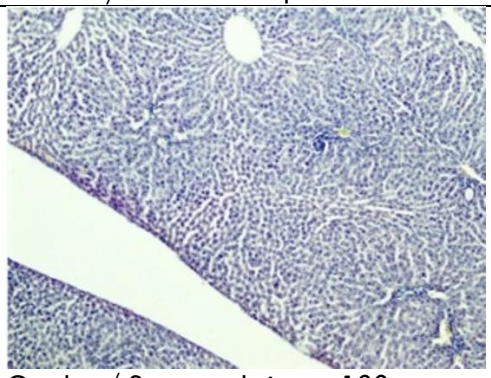
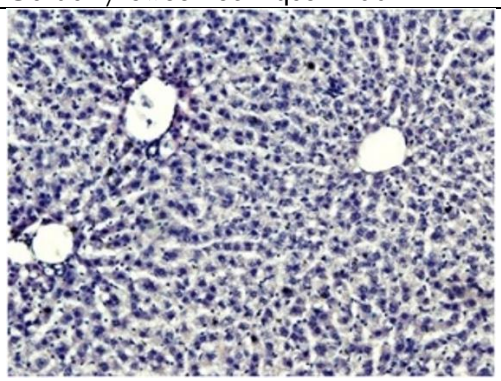
Histomorphologic analysis of liver tissue was performed using a Nikon Eclipse Ci research microscope (Nikon Instruments Inc., Americas). Micro-measurements were conducted with the integrated mechanical stage featuring graduated locator margins, built-in slide holder, and X-Y translator knobs. Histomorphological images were captured using the Nikon DS-Fi1c color camera and processed with NIS-Elements imaging software (Nikon Instruments Inc., Americas). Histological sections of liver parenchyma were analyzed and evaluated for various histological parameters, including fibrosis, cholestasis, steatosis, congestion, degeneration, and inflammation. Fibrosis was staged according to the Ishak system, inflammation was graded using the Ishak and Suzuki systems, cholestasis was scored using the system preferred by Dixon & Crawford, and steatosis was graded using the Kleiner-Brunt scores.

Results:

Microscopic examination was conducted on biopsies obtained from 3 SD rats in each of the 6 groups. Descriptive histological changes and representative histologic images observed at each time interval are presented in Table 2.

Table 2: Spectrum of Histological Changes after Hepatectomy

Time	Description of Histologic Changes	Histologic Appearances of Liver
30 Mins	No fibrosis, steatosis or cholestasis identified Acute infarct observed on reticulin stain	 Gordon and Sweet technique x 100
48 Hours	Moderate cytoplasmic vacuolization in 50% of the cells Disruption of cytoplasmic membrane Blurring of the cell-to-cell and cell-to-sinusoidal boundary Confluent necrosis involving 10% of parenchyma Necrosis extends to central veins and portal tracts Inflammatory changes at the infarcted area, with peripheral neutrophils	 H&E and Gordon / Sweet technique x 100
72 hours	Moderate cytoplasmic vacuolization in 50% of cells Disruption of cytoplasmic membrane Blurring of the cell-to-cell and cell-to-sinusoidal boundary Few lymphocytes in sinusoidal space, but no lytic necrosis Confluent necrosis involves 35% of parenchyma Necrosis extends to central veins and portal tracts	 H&E and Gordon / Sweet technique x 100

Time	Description of Histologic Changes	Histologic Appearances of Liver
7 days	Marked cytoplasmic vacuolization in 80% of the cells Disruption of cytoplasmic membrane Blurring of the cell-to-cell and cell-to-sinusoidal boundary Confluent necrosis has resolved Patchy portal inflammation involves 33% of portal tracts One portal tract shows granulomatous inflammation More lymphocytes in sinusoidal space, but no lytic necrosis	 Gordon / Sweet technique x 100.
11 days	Resolving cytoplasmic vacuolization at 30% of the cells Resolving disruption of cytoplasmic membrane Resolving blurring of the cell-to-cell and cell-to-sinusoidal boundary Lytic necrosis at the hepatic lobules at ~1focus/10x field Patchy portal inflammation (similar to day 7)	 Gordon / Sweet technique x100
14 days	Resolving cytoplasmic vacuolization at 20% of the cells Mild disruption of cytoplasmic membrane Mild blurring of the cell-to-cell and cell-to-sinusoidal boundary Lytic necrosis at the hepatic lobules at ~2foci /10x field Patchy portal inflammation (similar to day 7)	 Gordon / Sweet technique x 200

A morphometric evaluation of hepatocyte size (area) was performed to assess the progression of liver regeneration after 30% partial hepatectomy. The results showed a decrease in hepatocyte size, indicating the presence of smaller hepatocytes during the regeneration process three days after partial hepatectomy. The size of hepatocytes returned to normal levels seven days after partial hepatectomy, with a notable sharp decrease observed three days after the procedure (Figure 1).

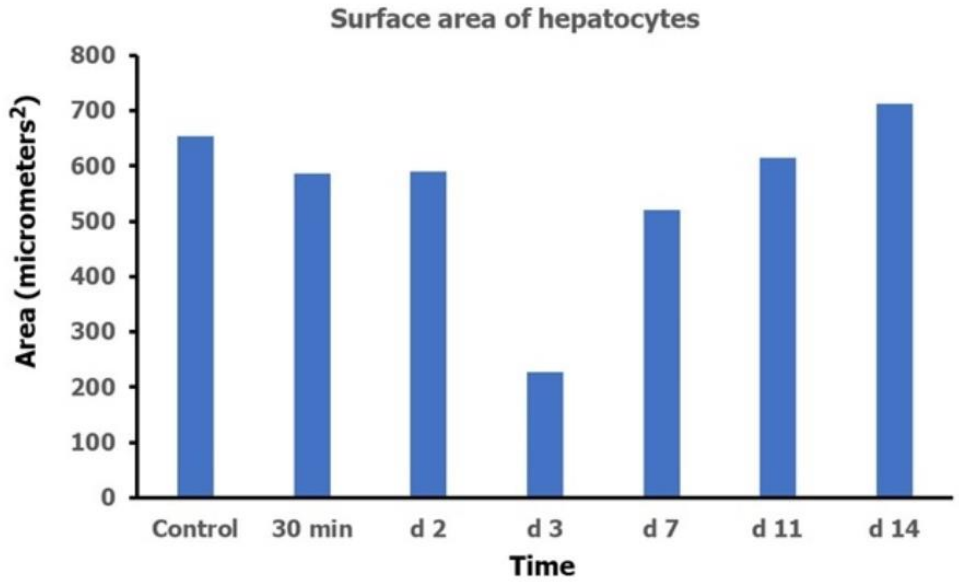


Figure 1: Graphic representation of hepatocyte size during the regeneration process.

Discussion

Through the pioneering work of Higgins and Anderson in 1931, we have understood some of the physiologic processes occurring in the FLR during regeneration.[10] Other researchers have partially elucidated signalling pathways by mitogenic growth factors and cytokines that induce DNA synthesis in hepatocytes, contributing to FLR regeneration.[15,16,17] However, there is still limited information on the histologic changes occurring in the FLR. This study has added to the existing knowledge on the histologic changes in the FLR.

It is clear that the regenerative processes differ between species. For example, liver regeneration begins at 24 hours in rats, 36 hours in mice, and 5-7 days in humans.[11] Nevertheless, animal studies provide an important contribution to the understanding of these processes and the information gleaned can be extrapolated to humans.

This study has helped us to understand the histologic changes that accompany the three regenerative phases: priming, progression, and termination.[9] During the first six hours of resection, the hepatocytes are in the priming phase preparing for cell cycle re-entry. This is marked by the release of pro-inflammatory cytokines, such as TNF, TGF-ß, IL-6, NF-κB, c-Jun, STAT3, and c-Myc.[9,18] In our experiments, the hepatocytes appeared microscopically quiescent during this priming, and no histologic changes were observed.

The second phase of FLR regeneration is progression, where there is stimulation of the complete cell cycle machinery, marked by active DNA replication and hepatocyte proliferation.[9,18,19] It has been shown in previous studies that this is an immune response, driven by interferon-gamma released from Natural Killer cells and T lymphocytes.[9] In our study, microscopic changes in this second phase were seen at 48 hours. This commenced with acute inflammatory changes at the periphery of the FLR, followed by granulomatous inflammation at 72 hours. It is clear that FLR inflow and immune competence are required during the progression phase in the critical period 48-72 hours post resection. From a clinical perspective, therefore, it is important that clinicians optimize patients' volume status to ensure proper liver perfusion and also to avoid drugs with anti-inflammatory and/or immune mediating function.[20] There are a few techniques used in clinical practice that aim to induce injury and/or stimulate inflammation at the FLR, while maintaining flow in order to encourage in-vivo regeneration.

Portal vein embolization, probably the most common, involves occlusion of portal venous flow to the hemi-liver intended for resection. This redirects flow to the contralateral side, leading to cellular hypertrophy and increasing the FLR size.[20] The theory behind this technique is that the combination of peri-portal inflammation in the emoblized liver and the diversion of portal venous blood to the FLR stimulate regeneration.[20,21,22]

Some authors have reported administering Yttrium-90 labelled microspeheres to the hemi-liver intended for resection resulting in ionizing radiation injury to the diseased hemi-liver[23,24]. This Yttrium-90 radioembolization maintains flow to the FLR, induces injury and inflammation at the diseased hemi-liver,

and has the theoretic advantage to simultaneously induce FLR growth and control tumour on the contralateral side,[20,24]

Surgical options also exist when there is metastatic disease on both sides of the liver. The first option is a two-stage hepatectomy with vein ligation, where the wedge resections performed to remove metastases from the FLR along with surgical ligation of the contralateral vein.[20] This maintains FLR perfusion and induces bilateral injury (ischemic injury to the ligated side and iatrogenic injury to the FLR), thereby accelerating regeneration. In a second stage operation, the surgeons would resect the hemi-liver with remnant metastases.[20,25]

Schlitt et al were first to describe the ALPPS (Associating Liver Partition and Portal vein ligation for Staged hepatectomy) technique.[20,26] The first stage in ALPPS is for the liver parenchyma to be divided along with surgical ligation of the portal vein supplying the hemi-liver to be resected (not hepatic artery or bile ducts).[26] The principle is that ALPPS results in more hepatic ischemia, injury and inflammation because both the portal vein and the rich intra-parenchymal anastomoses are interrupted, thereby resulting in faster FLR regeneration.[20,26,27] It is during the second staged operation, after FLR regeneration, that the hepatic artery and bile duct are interrupted and the liver resection completed.

Termination is final stage of the regeneration process, where hepatocyte proliferation stops after liver mass is restored.[9] In our experiments, this occurred around day 14 and was histologically represented by 20% cytoplasmic vacuolization as markers of recovery. The timing of this stage was similar to those in other animal studies.[28,29,30] Although our experiments have provided detailed insights into the histological changes during FLR regeneration, a future area for research is to correlate these histologic changes with function at the hepatocyte level, and various immune / inflammatory mediators that may modulate the regenerative process. This may be an area for future research.

Conclusion:

This study provides detailed insights into the progressive cascade of histologic changes during liver regeneration post-hepatectomy. The process is controlled by the inflammatory and immune responses post-injury and may be affected by many physiologic, iatrogenic, and pathologic processes. This is an area for future research.

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