Acceleration the Liver Regeneration by Hyperbaric Oxygen Therapy in Model Rabbit

HH Nahi, Zainab J. Malik, Karem Kadhem

Department of Surgical and Obstetric, Veterinary Medicine Collage, Al- Qasim Green University, Iraq.

*Corresponding author: malikzai87@gmail.com

Abstract

In this study, we were investigating the importance of therapy of the hyperbaric oxygenon the regeneration of liver. Sixteen mature rabbits of Iraqi breed were used. The animals divided equally into two groups (control and treated). The anesthetizing of animals was done by atropine sulphate (1mg /Kg B.W) intramuscularly; as premedication and 15 minutes later a mixture of xylazine hydrochloride (20mg /Kg B.W) and Ketamine hydrochloride (40 mg /Kg B.W) are giving intramuscularly. In control group the rabbits were anesthetized and preparation the site of operation was done in the upper part of abdomen. Surgical incision made and exposure of fourth lobe of the live. The liver handling by thump forceps and the stitches was done on the lobe. Three knots were tied and we used dissecting scissors in order to cut the tied lobe just distal to the suture. After that the wall of abdomen was re approximated with a running 3-0 Polyglactinsuture, then the skin closed with a running 2-0 polyamide suture. While in treated group the same procedure that mentioned above with hyperbaric oxygen therapy was done. The clinical parameters included temperature, respiratory, and heart rates, were withi acceptable limits post operatively in all animals in two groups. The intra-abdominal adhesions in different degrees of evaluations and occurred among liver, different organs and abdominal wall that more frequency in control group when compared with treated group. Histopathological Examinations was done by taken the liver biopsies on 15rd, and 30th, day postoperatively. The histopathological results of this study revealed that the liver regeneration more maturity in treated group than in control group.

Keywords: Hyperbaric oxygen therapy, Liver, Rabbit, Regeneration.

Introduction

The liver is essential to life and has multiple functions termed: glucidic, lipidic, proteinic and biliary; as well as involved in detoxification processes that added to regulation of hormonal metabolism. In addition, the liver processes reticular endothelial cells with putative macrophage-like which is in the storage of vitamins, small molecules and blood play an important role (1). Most veterinary sessions of high-pressure HBOT use pressures of 2.0 to 3.0 ATA (2), but therapy of low-pressure may be performed at 1.5 to 2.0 ATA.

However, the treatment time may be longer when lower pressure is used. The time of average treatment at 2 ATA is 45 to 60 minutes. Patients of Small animal are basically treated every 8 to 12 hours; but large animals have been treated up to six times in a 24-hour period, depending on the condition severity (3, 4). The aim of current study was accelerated the liver regeneration by of oxygen therapy

Materials

Anesthesia and Drugs

- Xylazine: Xyla 2% (Castenray, Holland.)
- Ketamine hydrochloride 5% (Pather. Londol .T.D.)
- Penicillin - Streptomycin: (Penoksia LA, Vilsan Ankara).

Hyperbaric oxygen chamber

Chambers of human HBOT are either multi place or mono place. However, in practices of veterinary can acquire previously owned mono place equipment from facilities of human in order to treat their patients. Hyperbaric hambers of Veterinary-specific are available too, with some being large enough for patients of ambulatory equine.
Reaching to hyperbaric oxygen as a treatment modality is currently limited due to the modest number of facilities with the appropriate equipment (2), there are all types are not available in Iraq, so that we modified autoclave to determine that same results and exactly have same results.

**Experimental Animal Group**

Twenty adult rabbits were selected from a herd Animal Resource of the College of Veterinary Medicine/Al-Qasim Green University. Rabbits were aged (3-10) months and weighted (1.5-2.5) Kg. Rabbits were kept in controlled environments throughout the experiment for observation and adaptation and were subsequently housed in pairs in (3×2) meters pens for the entire experiment.

**Experimental Design**

The Rabbits have been randomly divided to two groups (10 rabbits/group) as follows:

**First Group**: named group (A).

The rabbits of this group (which numbered from 1-10) have partial hepatotomy (2*1.5 cm).

**Second Group**: group (B).

In this group, the rabbits (which numbered from 11-20), that have partial hepatotomy was treated with hyperbaric oxygen therapy. After that, each group randomly divided equally to two subgroups for histopathology at 15th and 30th day respectively.

**Surgical Procedure**

Surgical Procedures: Resection of Hepatic was performed as described previously by Higgins and Anderson, 1931 (5). The anesthesia of rabbits was done by 0.8 mg/kg Acepromazine, 0.05 mg/kg Buprenorphine and 40 mg/kg of Ketamine intramuscular then a midline laparotomy was performed. Surgical incision was done in the upper part of abdomen (Fig. 1), and exposure of fourth lobe of the liver (Fig. 2), The handling of liver was done by using thump forceps (Fig. 3), then the lobe partial respected gently (2*1.5 cm) with a polyglacine 3-0 suture tie was placed underneath it and positioned as proximal to the origin of the lobe as possible (Fig. 4).

The both ends of the suture were tied over the liver lobe at its base near the inferior vena cava (Fig. 5). Three knots were tied and dissecting scissors were used to cut the tied lobe just distal to the suture. Then the wall of abdomen was re approximated with a running 3-0 Polyglactinesuture, and the closing of skin was done with a running 2-0 polyamide suture. (6, 7).

![Figure 1: The rabbits were anesthetized and preparation the site of operation was done in the upper part of abdomen](image1)

![Figure 2: The midline laparotomy was performed. Surgical incision was done in the upper part of abdomen and exposure of fourth lobe of the liver](image2)
Clinical Examination

The animals were examined physically and clinically for checking temperature, respiratory rate, heart rate, defecation and urination during a period of one week post operation.

Adhesion Evaluation

Adhesions were evaluated quantitatively and qualitatively at 15th and 30th days postoperatively. Evaluation was done depending upon two adhesion grading schemes:

Histopathological Examinations

Liver biopsies were taken on 15rd and 30th day postoperatively. Biopsies were obtained by using electrocautery. Biopsies were fixed in buffered formalin with concentration 10 %, then processed and embedded routinely in paraffin as blocks which were cut at 5-6 micrometer and finally stained by Hematoxyline and Eosin stain and then the examination done by light microscope.

Statistical Analysis

The results as means ± S.E. were expressed. The analysis of parametric data was done by two ways; the first one analysis of variance (ANOVA) which, continued with Least Significant Difference (L.S.D.), and (p> 0.05) was considered to be significant. Statistical Package for Social Sciences (SPSS) was used (8).
Results and Discussion

Clinical Examination

The results of the physical and clinical examination for checking the temperature, respiratory rate, heart rate, defecation and urination during first week post operation revealed that, slight elevation in temperature, respiratory rate and heart rate with normal defecation and urination in all animals. Significant convergence in results between the control group and treated group since the second day of post-operation, but early disappeared in treated group when compared with control group at 3rd and 5th days post-operation respectively, that may be due to increase the blood flow in operative area.

Beside that increase dilatation of blood vessels with increase permeability of capillaries was agreed with other workers (9) whom mentioned that there were no significant changes recorded in these clinical parameters before and after surgical operation. The animals’ activity which was represented by animal posture, motion, alert to the surrounding and the appetite in present study were not altered.

However, there were three animals in control group have normal appetite but limited activity during three days postoperatively; this is may be attributed to visceral pain or adhesion formation. The visceral pain during the first hours after surgical intervention or pain associated with adhesion formation postoperatively reported in previous researches (10, 11).

Adhesions

The intra-abdominal adhesions are showing more frequency in control group rather than in treated group in different degrees of evaluations and occurred among liver, different organs and abdominal wall. Quantitative evaluation (MSAS) (Table 1) showed that control group had large number (4 animals) of adhesion at grade 2, while the treated group had large number (8 animals) of adhesions at grade 0, (1 animals) at grade1 and (1animals) at grade 2 therefore the entrance to the abdominal cavity and ease of visualization of the abdominal organs were easy at treated group.

Qualitative evaluation (ATTS) (Table 2) showed that control group had grade 2 adhesions at (5 animals). While treated group had grade 0 adhesions at (8 animals).

Therefore, the adhesions in treated group had little effect and easy to perform adhesiolysis comparing with control group. Adhesions formation is part of normal healing process (12). One of the most potent stimuli for initiation of an inflammatory response and thus adhesions formation is surgical trauma. Routine surgical procedures involve various degree of tissue handling that initials tissue abrasion, desiccation, ischemia, bleeding, infection and exposure to foreign materials, any of these factors can initiate inflammatory responses which eventually lead to adhesion formation (13).

During day's five to ten, fibroblasts become aligned with the adhesion, while collagen deposition and organization advance. At two weeks later, the relatively few cells present are predominantly fibroblasts. 15th to 30th days after injury, the collagen fibrils become organized into discrete bundles interposed by fibrocytes and a few macrophages. Extensive well-defined adhesions are often covered by mesothelium and contain blood vessels and connective tissue fibers (14, 15).

A: Quantitative evaluation: Multiple sites adhesions scheme (MSAS) modified from (Ghahiri et al.) is used

<table>
<thead>
<tr>
<th>Degree of adhesion</th>
<th>Number of adhesion sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No adhesions</td>
</tr>
<tr>
<td>1</td>
<td>One adhesion sit</td>
</tr>
<tr>
<td>2</td>
<td>Two adhesion sites</td>
</tr>
<tr>
<td>3</td>
<td>Three adhesion sites</td>
</tr>
<tr>
<td>4</td>
<td>Four adhesion sites</td>
</tr>
</tbody>
</table>

B: Qualitative evaluation: Adhesive tissue tenacity schemes (ATTS) (Greene et al.) is used

<table>
<thead>
<tr>
<th>Degree of adhesion</th>
<th>Number of adhesion sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No adhesions</td>
</tr>
<tr>
<td>1</td>
<td>The adhesion fell a part</td>
</tr>
<tr>
<td>2</td>
<td>Adhesion lyses with traction</td>
</tr>
<tr>
<td>3</td>
<td>Adhesion lyses with blunt dissection</td>
</tr>
<tr>
<td>4</td>
<td>Adhesion lyses with sharp dissection</td>
</tr>
</tbody>
</table>
Table 1: Multiple sites adhesions schemes (MSAS).

<table>
<thead>
<tr>
<th>Table 1 Groups</th>
<th>Number of animals for each grade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade 0</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>Treated</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 2: Adhesion tissue tenacity schemes (ATTS)

<table>
<thead>
<tr>
<th>Table 2 Groups</th>
<th>Number of animals for each grade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade 0</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>Treated</td>
<td>8</td>
</tr>
</tbody>
</table>

Figure 6: Macroscopic view shows grade 2 adhesion (MSAS) among liver, omentum and abdominal wall in control group (A) and treated group (B)

Histopathological Findings

The histopathological findings of the liver after partial hepatectomy were as following:

Control Group

At 15th Day Post-operation:
The histopathological examination in control group at 15th day Post-operation showed marked coagulative necrosis of hepatocytes which characterized by inflammatory zone (Fig. 7), also there were neutrophils in dilated blood vessels and fibrin deposition in respected area with necrosis of hepatocytes (Fig. 8), other section showed widely dilated sinusoid and vacuolar degeneration of hepatocytes adjacent the respected area (Fig.9).

At 30th Day Post-operation
While, the control group at 30th day post-operation, showed granulation tissue invasion the necrotic area (Fig. 10). Another section showed that hepatocytes adjacent resected area had mild regeneration which characterized by polymorphic hyper-chromic nuclei compact together form mass without sinusoid extend to necrotic area (Fig. 11). In other slide, showed mild regeneration of hepatocytes (Fig. 12).

Treated Group

At 15th Day Post-operation
In group (A), there was granulation tissue which characterized by blood vessels and cellular FCT infiltrated with mononuclear cells (Fig. 13). Another section showed that regenerative activity of intact hepatocytes adjacent resected area which characterized by hyper chromatin of their nuclei with mitotic fingers, and there was dilated sinusoid and irregular architecture (Fig. 14). Another section showed extensive regeneration activity in hepatocytes adjacent respected part which forms nodular structure without central vein and sinusoids extend into the resected part (Fig.15).

At 30th Day Post-operation
Histopathological sections of treated group at 30th day post-operation, showed that normal structure of the hepatocytes in area far away from resected area with dark basophilic in their nuclei and some of cells have more nuclei (Fig.16). Another section, showed liver returned to the normal structure which consist from hepatocytes cord around central vein (Fig. 17). And normal hepatic architecture which represented by normal
lobules, CV and sinusoids (Fig. 18). The results of histopathological examinations of two groups at 15th day post-operation showed that, the treated group had less pathological findings, and the hepatocytes adjacent the resected area showed mild pathological changes, also early and extensive regeneration of hepatocytes than control group, all these results indicated that the hyperbaric oxygen therapy support adequate nutrient for all body that included liver in addition the oxidation glucose supply brain in few second while in natural condition adequate few minutes, this observation was described by other workers (16).

On other hands, the thermal heat of monopolar electrocautery device generate heat either directly (heater probe) or indirectly by tissue absorption, this heat leads to edema, coagulation of tissue proteins (17). There is formation of granulation tissue in necrotic area at Group (A) on 7th day, mayindicate improper environments in this area which need to attract fibroblasts and angioblast by growth factor which produce by microphages. Fibroblasts produce collagen fibers and angioblasts form capillary blood vessels to form granulation tissue and the degree of granulation tissue depend on degree of tissue destruction. The Presence of myofibroblasts in granulation tissue which originated from mature fibroblasts indicated, there is sever destruction and these cells attempt to contract the affected area due to their active as smooth muscle cells. This idea was agreed with (18).

In present study, the liver regeneration appeared early and extensive in group (C) comparing to other groups, this is due to little hepatic damage and necrosis during ultra-surgical technique. This finding coincides with others (19), whom said that the liver regeneration occurred rapidly after the right lobe's resection of humans' liver. In addition to that, (20), explain that the hepatocyte, regeneration occurs when the injury affecting the hepatocytes only; while repair of the liver occurs when injury affecting hepatocyte with stromal tissue.

Also (21) in their study on the normal human liver found that mitosis appear at 10 and 35 days and the histopathological findings were; cell with basophilic cytoplasm or two nuclei and the liver remnant was morphologically normal at 30th post-operation.
Figure 9: Histopathological section of liver related to control group, at the 15th day post operation; Shows dilated sinusoid (A) and vacuolar degeneration of hepatocytes (B) (H&E 40X)

Figure 10: Histopathological section of liver related control group, at the 30th day post operation; shows granulation tissue invasion the necrotic area (arrow) (H&E 40X)

Figure 11: Histopathological section of liver related to control group, at the 30th day post operation; Shows mild regeneration of hepatocytes adjacent respected area (arrow) (H&E 40X)

Figure 12: Histopathological section of liver related to control group, at the 30th day post operation; Shows mild regeneration of hepatocytes (arrow) (H&E 40X)
Figure 13: Histopathological section of liver related to treated group, at 15th day post operation; there is extensive regeneration activity in hepatocytes adjacent respected area (H&E 40X)

Figure 14: Histopathological section of liver related to treated group, at 15th day post operation; shows granulation tissue infiltrated with mononuclear cells (A) and appear of my fibroblast (B) (H&E 10X)

Figure 15: Histopathological section of liver related to treated group, at 15th day post operation; the intact hepatocytes adjacent respected area shows regenerative activity (arrow) (H&E 100X)

Figure 16: Histopathological section of liver related to treated group, at 30th day post operation; the hepatocytes in area far away from respected part shows dark basophilic nuclei (A) and some of cells have more nuclei with mitosis (B) (H & E 40X)
Figure 17: Histopathological section of liver related to treated group, at 30th day post operation; liver returned to the normal structure which consists from hepatocytes cord around the central vein (arrow) (H&E 100X)

Figure 18: Histopathological section of liver related to treated group, at 30th day post operation; shows normal hepatic architecture which represented by normal lobules, CV (A) and sinusoids (B) (H&E100X)

References


