Hyperbaric Oxygen (HBO) Therapy after Partial Hepatectomy: An Experimental Study on Oxidative Stress in Rats

by ***Mehrdad Bohloli[1], Hafize Uzun[2], Erman Aytac[1], Akin Savas Toklu[4], Melih Paksoy[1], Haydar Durak[4] & Turgut Ipek[1]***

[1]Department of General Surgery,

[2]Department of Biochemistry, and

[3]Department of Pathology, Istanbul University Cerrahpasa Medical Faculty, Istanbul, Turkey

[4]Department of Hyperbaric Medicine, Istanbul University Istanbul Medical Faculty, Istanbul, Turkey

Summary

In this study, we evaluated the effects of HBO therapy on oxidant and antioxidant status, and some histopathological parameters after partial hepatectomy in rats. There were three main experimental groups. The first group was sham (S) group (n=12), the second group was partial hepatectomized (PH) group (n=24) and the third group was after partial hepatectomy HBO-treated group (PH+HBO) (n=24). The HBO therapy was started the same day as partial hepatectomy for the PH+HBO group. Three subgroups (n=8) were assigned from the PH and HBO groups. The first, the second and the third subgroups were selected randomly, separated from the main groups and killed 2 days, 4 days and 7 days after partial hepatectomy respectively. Randomized selection from the main experimental groups and killing of the animals were done on the same day. Malondialdehyde (MDA), glutathione (GSH), nitric oxide (NO), mitotic index (MI), proliferating cell nuclear antigen (PCNA) levels and mean of cells with double nuclei were assessed. In the PH+HBO group, MDA and NO levels were decreased and MI, PCNA, GSH levels were increased significantly compared to the PH group. HBO therapy could activate antioxidant response and reduce oxidant activity after partial hepatectomy.

Introduction

Partial hepatectomy is performed for treating liver pathologies especially for the curative treatment of hepatic malignancies and for living-donor liver transplantation *(Bedirli et al, 2005)*. Hepatocytes undergo cell division after injury or surgical removal of a portion of the liver. The liver has regenerative capacity for restoring it to normal size. Many different cell types such as hepatoctyes, biliary epithelial cells, fenestrated endothelial cells and Kupffer cells are active in hepatic regeneration *(Michalopouos and DeFrances, 1997)*. Studies of the partial hepatectomy model have shown that initiation of the regenerative response depends on many factors *(Bedirli et al, 2005)*. Endocrine and paracrine actions of growth factors, activation of specific proto-oncogenes and transcription factors are important during regeneration *(Webber et al, 1993)*.

Hyperbaric oxygen (HBO) therapy has beneficial effects on many surgical problems such as non-healing wounds, clostridial myonecrosis, radiation injury, osteomyelitis, crush injury, compartment syndrome and acute necrotizing infections *(Morello et al, 1990; Bakker, 2000)*. It has been suggested that HBO therapy can inhibit pro - inflammatory cytokines activity. The increased oxygen concentration and increased partial pressure of oxygen during HBO therapy provides more oxygen for the whole

**Correspondence:* Dr. Hafize Uzun, Ph D.
Istanbul Universitesi Cerrahpasa Tip Fakultesi, Temel Bilimler Binasi-Biyokimya Anabilim Dali, 34098 Cerrahpasa, Istanbul, Turkey
Tel: +90 212 414 30 56
Fax: +90 212 633 29 87
E-mail: huzun59@hotmail.com

body *(Gulec et al, 2004)*. Increased oxygen is needed by the dividing cells to rebuild the lost hepatic tissue after partial hepatectomy *(Yoshioka et al, 1998)*. Recently it has been reported that arterializations of the portal vein with anastomosis between arteries which are near the portal vein has beneficial effects on hepatic energy metabolism and liver regeneration due to increased blood supply and oxygen value. It has also been shown that hyperbaric oxygenation after portal vein occlusion supports liver regeneration *(Shimizu et al, 2000; Uwagawa et al, 2001)*. Partial hepatectomy could cause oxidative stress like other surgical procedures (*Miyake et al, 2002*). HBO therapy before 15 % partial hepatectomy supported subsequent liver regeneration and decreased high tissue-lipid peroxidation and decreased levels of serum aminotransferases *(Kurir et al, 2004)*. Different techniques are under investigation about reducing tissue damage after surgery and for improving hepatic regeneration (*Hortelano et al 1995; Chen et al, 1996; Kapan M et al, 1996*).

Mitotic index (MI) and proliferating cell nuclear antigen (PCNA) have been used as experimental parameters to monitoring liver regeneration (*Shimizu et al, 1999*).

The data on the effects of HBO therapy after partial hepatectomy on oxidative stress is limited. The effects of HBO therapy on oxidant/antioxidant status and various histological parameters at different time periods following partial hepatectomy were evaluated in this study.

Materials and Methods

This study was conducted according to the guidelines of the animal care review board of Istanbul University, Cerrahpasa Medical Faculty, in accordance with National legislation and The Council Directive of the European Communities on the Protection of Animals Used for Experimental and Other Scientific Purposes *(L358/1, November 24, 1986)*. 60 adult male Sprague-Dawley rats weighing between 210-380 g were taken from the Cerrahpasa Medical Faculty Experimental Animal Production and Research Laboratory. The rats were kept in standard colony cages (15X25X40cm) (4 rats per cage) under controlled conditions including temperature (28 °C), light (10 h light: 14 h dark), humidity (50 F 55%). The animals were fed with standard rat chow and tap water *ad libitum* pre- and post-operatively. No drugs or pain relief regimen were given to the rats post-operatively, because it has been shown that many anti-inflammatory drugs affect liver regeneration and functions, and could affect our results if used *(Gershbein and Pedroso,1985)*. The rats were divided into three main experimental groups. The first group was sham (S) group (n=12), the second group was partial hepatectomized (PH) group (n=24) and the third group was after partial hepatectomy HBO-treated group (PH+HBO) (n=24). The HBO therapy started when the partial hepatectomy operations were finished and the animals had recovered from the effects of anesthesia. Three subgroups (n=8) were assigned from both PH and PH+HBO groups. The first subgroup was selected randomly, separated from the main groups and killed 2 days after partial hepatectomy. The second subgroup was selected randomly, separated from the main groups and killed 4 days after partial hepatectomy. The third subgroup was selected randomly, separated from the main groups and killed 7 days after partial hepatectomy. The randomized selection from the main experimental groups and killing of the animals were don on the same day. The rats were killed by decapitation.

Thoracotomy and laparotomy were performed immediately after decapitation. Total blood of 6 ml was collected from the right ventricle with an injector and liver tissue samples were collected immediately. The liver samples were immediately immersed in liquid nitrogen and stored at -70 °C until being processed for biochemical investigation. To determine MI levels, some other parts of these liver samples were fixed in 10% formaldehyde solution.

Surgical Procedure

The operations were performed in groups of rats between 9 am and 12 noon in order to standardize the effects of diurnal changes. The rats were given only ether anesthesia in a closed jar. Induction time took 2-3 minutes. The abdominal area was cleansed with Batticon® (Povidine iodine) solution after shaving. A median abdominal incision was performed. The left and median lobes of the liver were exposed with the standard 70% hepatectomy technique *(Waynforth and Flecknell, 1992)*. The peduncle of the left and median lobe was resected with 4/0 silk initially. The right and caudate lobes of the liver were left in place in all of the rats. The abdominal incision was closed with 2/0 silk continuous sutures as a single layer. All of the operations were performed under clean but not sterile conditions. All operations for performing partial hepatectomy were started with sterile surgical tools for every rat but during operation, sterility was not kept because of keeping the rat in an appropriate position. Neither operative nor postoperative loss occurred in the groups. The body weights of the rats were recorded after the hepatectomy and at the end of the HBO therapy.

Hyperbaric Oxygen Therapy

HBO therapy was performed for seven days on the PH+HBO group. HBO treatment was performed in the one-compartment experimental pressure room with 0.28 m^3 volume at the Istanbul Medical Faculty, Department of Diving and Hyperbaric Medicine. HBO treatment commenced about 3 hours after the operation. The treatment protocol was 4 sessions in the 1st and 2nd days, 3 sessions in the 3rd and 4th days, 2 sessions in the 5th, 6th and 7th days with equal intervals. Each session lasted 80 minutes and consisted of ventilation for 10 minutes (for 100% oxygenation of the pressure room), diving at 50 feet (ca. 15.4 m) for 60 min (chamber pressure, 2.4 atmospheres) and surfacing for 10 minutes. Diving phase (compression) in HBO lasts for 5-10 minutes generally.

Biochemical Procedure

Heparinized blood samples taken from the right ventricle after thoracotomy were centrifuged at 2500 rpm at + 40 °C for 10 min to extract the plasma. The erythrocytes were washed by centrifuge with sterile saline (0.9% NaCl) at + 40 °C at 2500 x g for 15 min. This was repeated 3 times before the determination of GSH levels in the washed erythrocytes. Concentrations of plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), total plasma protein and albumin in plasma were studied on the same day with partial hepatectomy. The remaining plasma and liver tissue were stored at – 70°C until biochemical analysis for GSH, NO and MDA. At the time of study, liver tissue samples were weighed and homogenized with 0.15M KCL. The tissue homogenates were sonicated twice with a 30 seconds interval at moderate intensity. After completion of sonication, the homogenates for MDA measurement were centrifuged at 3000 rpm for 10 min whereas those prepared for NO determination were centrifuged at 15000 rpm for 15 min to obtain supernatants. All procedures were performed with ice cooling.

Assay of Plasma Aspartate Aminotransferase, Alanine Aminotransferase, Plasma Albumin and Total Protein Levels

Plasma aspartate aminotransferase (AST), alanine aminotransferase, (ALT), total plasma protein and albumin levels were measured by enzymatic methods using commercial kits *(Olympus, Hamburg, Germany)* on an Olympus AU800 analyzer.

Assay of Glutathione

Reduced glutathione (GSH) concentration was determined according to the method of Beutler et al. using metaphosphoric acid for protein precipitation and 5-5´-dithiobis-2-nitrobenzoic acid for color development *(Buetler et al, 1963)*. Erythrocyte and tissue GSH concentrations were expressed as mg/g Hb in erythrocytes. Hemoglobin concentration was determined by the cyanamethemoglobin method *(Fairbanks and Klee, 1986)*.

Assay of Malondialdehyde

Lipid peroxidation was ascertained by the formation of malondialdehyde (MDA), which was estimated by the thiobarbituric acid method (*Angel et al, 1988*). One volume of sample was mixed thoroughly with two volumes of a solution of trichloracetic acid (TCA) (30%), thiobarbituric acid (TBA) (0.75%) and 5M hydrochloric acid (HCl). The tubes were placed in boiling water for 15 minutes and centrifuged at 3000 rpm for 10 minutes. The supernatant was read at 535 nm in a spectrophotometer. The amount of MDA was calculated using an extinction coefficient (1,56x105 M-1 .cm-1). Plasma and tissue MDA levels were expressed as nmol/ml and nmol/100mg protein, respectively. Protein concentration was determined by the Lowry method *(Lowry et al, 1951).*

Assay of Nitric Oxide

Nitric Oxide (NO) was measured as its stable metabolites nitrate (NO_3^-) and nitrite (NO_2^-). Nitrate was first reduced by nitrate reductase to nitrite and then nitrite was determined spectrophotometrically by the Griess reaction *(Green et al, 1982), (Roche, Cat No 1 756 281).* Plasma and tissue NO concentrations were expressed as µmol/L and µmol/g wet tissue, respectively.

Hepatocyte Mitotic Index

Mitotic index (MI) is defined as the number of dividing hepatocytes. In an attempt to determine MI and PCNA values, a portion of these liver samples was fixed in 10% formaldehyde solution. The tissue samples were embedded in paraffin blocks, cut with microtome to 3-5 micron thickness, placed on the slides, numbered, and stained with hemotoxylineosin. The stained samples were examined under a light microscope *(Olympus BX50, Tokyo)* with 400x magnification. The MI for each slide was calculated by means of the number of mitotic figures in 10 different fields.

Proliferating Cell Nuclear Antigen Labelling

Proliferating cell nuclear antigen (PCNA) is a cell cycle nuclear protein (molecular weight, 36 kDa) that is expressed in the late G1 and throughout the S-phase of the mitotic cycle. It has been widely used to mark cells of the S phase. Additionally, PCNA synthesis can be increased by growth factors *both in vivo* and *in vitro*. The amount of PCNA expression correlates with the degree of cell proliferation. Tissue sections on the underwent antigen retrieval via 20 minutes in a 800W microwave oven in citrate buffer at pH 6 after deparaffinization *(Hall et al, 1990).* Predilusion was done with PCNA antibodies *(Dako Glostrup, Denmark)*, (Labeled avidin-biotin complex technique), with chromogen (3-amino-9-ethylcarbozole) and heamatoxylin as contrast stain. The slides were examined under a light microscope *(Olympus BX50, Tokyo)* with 400x magnification. The percentages of the cells stained with immunohistochemical PCNA staining were calculated by counting the stained cells in 10 different fields for each individual slide.

Means of Cells with Double Nuclei

Cells with double nuclei can be seen in liver tissue under normal conditions but cells with double nuclei are also seen regenerating liver tissue at a higher rate than normal. Mean of cells with double nuclei and hyperchromatic nucleus were determined by calculating the arithmetic mean of the cells with double nuclei by counting the stained cells in 10 different fields for each individual rat under 400 x magnifications *(Kapan et al, 1996).*

Statistical Analysis

All data are expressed as means and standard deviations (means ± SD) and 95% confidence intervals. Data were compared between groups using one-way ANOVA (Scheffe's test was made as a post-hoc test). Student's *t* test was used for comparing weights of the rats after HBO therapy. SPSS 12.0 *(SPSS: Statistical Package for Social Sciences)* was used for assessing the significance of differences between groups. $P < 0.05$ was considered significant.

Results

Plasma MDA, plasma NO, erythrocytic GSH, plasma AST, plasma ALT, plasma total protein, plasma albumin, liver tissue GSH, liver tissue MDA and liver tissue NO levels of all experimental groups are summarized together in Table 1. Significant values were determined between the S (sham) group and the subgroups, which were killed on the same day.

PH+HBO subgroups plasma and liver tissue MDA levels were significantly lower than PH subgroups, which were killed 4 days and 7 days after PH ($p<0.001$). PH+HBO groups were becoming significantly lower with time compared to PH groups ($p<0.001$). PH+HBO groups NO levels were lower than PH groups, which were killed 4 days and 7 days after partial hepatectomy ($p<0.001$). The PH group erythrocytic GSH levels were decreased significantly compared to the S group ($p<0.001$). Erythrocytic GSH levels of the PH+HBO subgroup which were killed 2 days after partial hepatectomy were significantly lower than the S group ($p<0.001$) but PH+HBO subgroup which were killed 7 days after partial hepatectomy were significantly higher than the S group ($p<0.001$). AST and ALT levels of the PH+HBO subgroup, which were killed 2 days after, were higher than the PH subgroup ($p<0.05$), but 4 days and 7 days after partial hepatectomy, the PH group levels were significantly higher than the

Table 1. Plasma MDA, plasma NO, erythrocytic GSH, plasma AST, plasma ALT, plasma total protein, plasma albumin, liver tissue MDA and liver tissue NO levels of all experimental groups are summarized together below in the table. Significant values were determined between S group and the subgroups which were killed on the same day.

		1st. Subgroup (the 2-day group)		**2nd. Subgroup** (the 4-day group)		**3rd. Subgroup** (the 7-day group)	
	S group	**PH group**	**PH+HBO group**	PH group	PH+HBO group	**PH group**	**PH+HBO group**
Plasma MDA levels (nmol/mg protein)	2.10±0.21	**6.4±0.3*** **	**6.24±0.3*** **	7.01±0.34*** **	4.7±0.3*** §§§	**7.35±0.49*** **	**4.4±0.3*** ** §§§
Liver tissue MDA levels (nmol/mg protein)	1.11±0.01	**128±7.63*** **	**123±14.43*** **	134.85±6.71*** **	65.75±5.73*** §§§	**133.16±12.48*** **	**61.13±4.05*** ** §§§
Erythrocytic GSH levels (mg/g Hemoglobin)	3.71±0.16	**2.46±0.35*** **	**2.53±0.32*** **	2.03±0.3*** **	4.3±0.33§§§	**2.07±0.26*** **	**4.46±0.35*** ** §§§
Plasma NO levels (micromol/L)	21.21±2.62	**76.24±4.01*** **	**76.24±3.15*** **	79.77±3.47*** **	66.36±5.41*** §§§	**81.63±3.61*** **	**56.45±3.79*** ** §§§
Liver tissue NO levels (micromol/g wet tissue)	0.47±0.11	**0.71±0.22*** **	**0.66±0.16*** *	0.8±0.11*** **	0.56±0.21 §§§	**0.73±0.27*** **	**0.54±0.12** §§§
AST levels (U/L)	36.84±5.12	**720±503.65*** **	**1244.38±2165*** ** §	1378.85±364.94*** **	185.5±40.03*** §§§	**1030.5±307*** **	**168.25±8.48*** **
ALT (U/L)	26±4.98	**664.5±556*** **	**779.5±142.43*** ** §§	1093.85±364.94*** **	53.75±4.71*** §§§	**892.67±369*** **	**59.13±344*** ** §§
Total protein (g/L)	7.39±0.29	**6.96±0.6**	**5.55±0.12***	6.7±0.62	6.89±0.35	**6.97±2.08**	**7.26±0.31**
Albumin (g/L)	3.27±0.11	**3.95±0.9**	**2.33±0.23**	3.23±1.05	2.94±016	**3.55±2.23**	**3.2±0.23**

Significant differences between S group and other group defined with (*):
(*): $p<0,05$, (**): $p<0,01$, (***):$p<0,001$
Significant differences between PH and PH+HBO groups defined with (§):
(§): $p<0,05$, (§§): $p<0,01$, (§§§):$p<0,001$

Table 2: Means of cells with double nuclei, mitotic index (MI) and proliferating cell nuclear antigen labelling values of our experimental groups are summarized together below in the table. Significant values were determined between the subgroups which were killed on the same day.

	1st.Subgroup		**2nd. Subgroup**		**3rd. Subgroup**	
	PH group	**PH+HBO group**	PH group	PH+HBO group	**PH group**	**PH+HBO group**
Means of cells with double nuclei	**5±2.87**	**7.03±2.9**	10.23	8.32±3.71	**6.28±3.92**	**4.11±1.08**
MI	**0.69±0.3**	**4.83±2.24§§§**	0.22±0.1	3.8±1.77§§§	**0.34±1.77**	**0.61±0.04**
PCNA labelling	**2.52±1.53**	**63.78±11.59§§§**	1.41±0.3	69.3±19.09§§§	**7.11±0.3**	**4±0.9§**

Significant differences between PH and PH+HBO groups defined with (§):
(§): p<0,05, (§§): p<0,01, (§§§):p<0,001

Table 3: Differences between the weights of the rats after hepatectomy and before sacrifice. Each value represents the mean ± S.D.

	1st.Subgroup		**2nd. Subgroup**		**3rd. Subgroup**	
	PH group	**PH+HBO group**	PH group	PH+HBO group	**PH group**	**PH+HBO group**
Weights after PH	**308.5±32.85**	**284.84±31.97**	295**±31.61**	299.5**±44.5**	**294.5±11.55**	**274.62±41.02**
Weights before sacricifice	**304.25±32.15**	**288.25±32**	286.37**±31.36**	299.62**±43.96**	**288.75±13.46**	**281.87±40.82**
t	**0.84**	**0.10**	1.38	0.97	**3.24***	**2.66***

t, t- values according to paired-*t* test.
* Statistically significant decrease in weight of the untreated rats (*p<0.05*).
Differences between the weights of the rats after hepatectomy and before killing of rats. Each value represents the mean ± S.D.

PH+HBO group (p<0.05).
Means of cells with double nuclei, mitotic index (MI) and proliferating cell nuclear antigen labelling (PCNA) values of our experimental groups are summarized in Table 2. Significant differences were determined between the 5 groups an the other subgroups which were killed on the same day.
There were no differences between means of cells with double nuclei between experimental groups. The MI was significantly higher in the PH+HBO group 2 and 4 days after partial hepatectomy compared to the PH (only) groups (p<0.001). (Figure 1). PCNA levels of PH+HBO group were significantly higher than PH group 2 days, 4 days (P<0.001). In contrast, the PCNA level was highest in the remnant liver tissues of untreated rats on 7 days after hepatectomy (p<0.05) after partial hepatectomy. The body weights of PH+HBO group rats did not change; on the other hand the body weights of PH group rats were decreased significantly 4 and 7 days after hepatectomy (Table3).

Discussion

It has been shown that hepatectomy increases lipid peroxidation, which can cause liver injury *(Bilzer and Gerbes, 2000; Chen et al, 1996; Kaplowitz, 2000).*

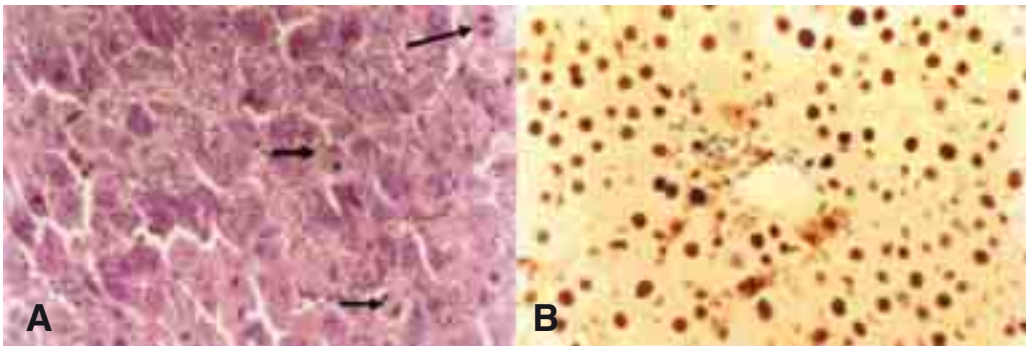

Figure 1. Mitotic index in the remaining rat liver after hepatectomy.
A: High mitotic index in the liver tissues of rats treated with HBO for 2 days. Arrows show mitosis. B: Low mitotic index in the liver tissues of untreated rats 2 days after hepatectomy. Specimens were stained by hematoxylin-eosin. Images were taken with Olympus BX50 light microscope *(high power field 400X magnification).* Scale bars in planes A and B are 100 µm.

The effects of HBO on oxygen free radicals are conflicting. Some researchers suggest that oxygen free radicals increase during HBO treatment but others proposed that HBO reduces the production of oxygen free radicals *(Narkowicz et al, 1993; Kudchodkar et al, 2000).*

The duration and timing of HBO treatment is important for a successful therapy *(Buras, 2000).* The procedure pressure never exceeds 3 atmospheres and usually does not last longer than 90 minutes for safety reasons. Free radicals may accumulate and cause oxygen toxicity *(Kimball et al, 2000).* Kurir at al. suggested that HBO pretreatment would be beneficial for rat liver regeneration after partial hepatectomy *(Kurir et al, 2004).* In our results on plasma and liver tissue, MDA levels were getting higher after partial hepatectomy but HBO therapy decreased MDA levels. The elevated MDA levels can depend on many conditions such as ischemia, acidosis, cellular damage and rate of mitosis *(Bilzer and Gerbes, 2000; Koudelova and Mourek, 1994; Patockova et al, 2003; Hortelano et al, 1995).* According to this data we suggest that HBO treatment may reduce oxidative stress and decrease liver damage after hepatic resection. Hyperbaric oxygen provides more oxygenation in the whole body. Increased tissue oxygen could support regenerative events by enhancing the growth of fibroblasts, collagen formation, angiogenesis etc. *(Yasar et al, 2003).*

As an antioxidant parameter we investigated erythrocytic glutathione (GSH) levels. GSH removes the toxic substances from the environment and protects the tissue from harmful substances after biotransformation. High GSH activity protects the cells from oxidative damage by inhibiting lipid peroxidation *(Kaplowitz, 2000).* HBO treatment increased the erythrocytic GSH level as shown in our results. Some studies demonstrated that antioxidant enzymes could be increased after hyperoxia *(Dennog et al, 1999).* In another study, HBO therapy increased GSH levels of the lung tissue (*Kimball et al, 2000*). MDA and GSH were suggested as useful markers in monitoring therapy of the liver pathologies *(Luguercio and Federico, 2002; Shen et al, 2000).* According to this data, we suggest that HBO treatment could support antioxidant response via accumulating GSH production.

AST and ALT are also regarded as hepatic necrosis parameters, increasing proportionally with the extent of hepatic necrosis *(Siddique et al, 2004).* In our study, the levels of AST and ALT were lower in the HBO treated group. HBO could decrease the tissue necrosis after hepatic resections. When we

studied the NO levels in our study, we found they were elevated at the same time with oxidan parameters. NO could initiate production of reactive oxygen species after partial hepatectomy and during liver regeneration. NO can mediate a number of physiological and pathological reactions involving the liver *(Li and Billiar, 1999)*. The role of NO seems to be controversial because in some models of inflammation, it has been shown that tissue dysfunction or injury could occur after inhibition of NO *(Kubes and McCafferty, 2000)*. However, high production of NO has been suggested as a cause of tissue injury under certain circumstances, perhaps through the generation of potent free radicals. Studies were not in consensus as to whether NO was cytotoxic or cytoprotective. It may act both as a cytotoxic agent and a cytoprotective agent, the main determinants being its concentration and the environment (*Hunt and Goldin, 1992; Werner et al, 1998)*.

In this study, proliferating cell nuclear antigen (PCNA) labeling, mitotic index (MI) and mean of cells with double nuclei were also determined to evaluate histological differences due to HBO therapy after partial hepatectomy. We found that the PCNA value was about 65% in the 2nd day after resection and made a peak in the 4th day reaching 70%. These values were compatible with the literature *(Eguchi et al, 1999)*. Additionally, there was a significant difference compared to the PH group. The highest MI values were found in the PH+HBO group 2 and 4 days after partial hepatectomy.

In conclusion, HBO therapy may protect the remaining liver tissue after partial hepatectomy and may support liver regeneration by reducing oxidative stress. However, we need more information for the exact evaluation of the effects of HBO therapy on hepatic regeneration**.**

References

Angel MF, Ramasastry SS, Swartz WM, Narayanan K, Kuhns DB, RE Basford & JW Futrell. The clinical relationship between free radicals and degrees of ischemia: evidence for tissue intolerance of marginal perfusion. Plast. Reconstr. Surg. 1988, *81*, 233-259.

Bakker DJ. Hyperbaric oxygen therapy and the diabetic foot. Diabetes Metab. Res. Rev. (Suppl). 2000, 16, *1*, 55-58.

Bedirli A, Kerem M, Pasaoglu H, Erdem O, E Ofluoglu & O Sakrak. Effects of ischemic preconditioning on regenerative capacity of hepatocyte in the ischemically damaged rat livers. J. Surg. Res. 2005, *125(1)*, 42-48.

Bilzer M & Gerbes AL. Preservation injury of the liver: mechanisms and novel therapeutic strategies. J. Hepatol. 2000, *32*,508-515.

Buetler E, O Duren & M Kelly. Improved method for the determination of blood glutathione. J. lab. Clin. Med. 1963, *51*, 882-888.

Buras J. Basic mechanisms of hyperbaric oxygen in the treatment of ischemia reperfusion injury. Intern. Anesth. Clin. 2000, *38*, 91-109.

C*hen MF, Chen HM, SW Ueng & MH Shyr*. Hyperbaric oxygen pretreatment attenuates hepatic reperfusion injury. Liver 1996, *18*, 110-127.

Dennog C, Radermacher P, YA Barnett & G Speit. Antioxidant status in humans after exposure to hyperbaric oxygen. Mutation Research 1999, *428*,83–89.

Eguchi S, Okudaira S, Azuma T, Ohno Y, H Fujioka & J Furui. Changes in liver regenerative factors in a case of living-related liver transplantation. Clin. Transplant. 1999, *13*, 536-546.

Fairbanks V & Klee GG. Biochemical aspects of hematology. Textbook of clinical chemistry. N.W.Ttietz (ed). W.B. Saunders Company, Philadelphia, 1986, pp 1532-1534.

Gershbein LL, Pedroso AF. Action of drugs and chemical agents on rat liver regeneration. Drug Chem Toxicol. 1985, *8(3)*, 125-43.

Gulec B, Yasar M, Yildiz S, Oter S, Akay C, S Deveci & D Sen. Effect of hyperbaric oxygen on experimental acute distal colitis. Physiol. Res. 2004, 53, *5*, 493-499.

Green LC, Wagner DA, Glogowski J, Skipper PL, JS Wishnok & SR Tennenbaum. Analysis of

nitrate, nitrite and (15 N) nitrate in biological fluids. Anal. Biochem. 1982, *126*, 131-138.

Hall PA, Levison DA, Wods AL, Yu CC, DB Kellock & JA Watkins. Proliferating cell nuclear antigen (PCNA) immunolocalization in paraffin sections. An index of cell proliferation with evidence of deregulated expression in some neoplasm. J. Pathol. 1990, *162*, 285-294.

Hortelano S, Dewez B, Genaro AM, MJ Diaz-Guerra & J Bosca. Nitric Oxide released in regenerating liver after partial hepatectomy. Hepatology 1995, *21*, 776-786.

Hunt NC & Goldin RD. Nitric oxide production by monocytes in alcoholic liver disease. J. Hepatol. 1992, *14*, 146-150.

Kapan M, Ipek T, Sad A, S Goksel & F Sirin. Effects of cyclosporin and somatostatin on liver regeneration after partial hepatectomy in rats. Eur. Surg. Res. 1996, 28, *4*, 262-269.

Kaplowitz N. Mechanisms of liver injury. J. Hepatol. 2000, *32*, 39-47.

Kimball RE, Reddy K, Peirce TH, Schwartz LW, MG Mustafa & CE Cross. Oxygen toxicity: augmentation of antioxidant defense mechanisms in rat lung. Am. J. Physiol. 2000, *230*, 1425-1431.

Koudelova J & Mourek J. The lipid peroxidation in various parts of the rat brain: effect of age, hypoxia and hyperoxia. Physiol. Res. 1994, 43, *3*, 169-173.

Kubes P & McCafferty DM. Nitric Oxide and intestinal inflammation. Am. J. Med. 2000, *109*, 150-158.

Kudchodkar BJ, Wilson J, A Lacko & L Dory. Hyperbaric oxygen reduces the progression and accelerates the regression of atherosclerosis in rabbits. Arteriosclerosis Throm. Vasc. Biol. 2000, *20*, 1637-1650.

Kurir TT, Markotic A, Katalinic V, Bozanic D, Cikes V, Zemunik T, Modun D, Rincic J, Boraska V, Bota B, I Salamunic & S Radic. Effect of hyperbaric oxygenation on the regeneration of the liver after partial hepatectomy in rats. Braz. J. Med. Biol. Res. 2004, *37*, 1231-1237.

Li J & Billiar TR. The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. Am. J. Physiol. 1999, *276*, 1069-1073.

Lowry OH, Rosebrough NJ, AL Farr & RJ Randall. Protein measurement with folin phenol reagent. J. Biol. Chem. 1951, *193*, 265-266.

Luguercio CV & Federico A. Oxidative stress in viral and alcoholic hepatitis. Free Radi. Biol. Med. 2002, *34*, 1-10.

Michalopouos GK & DeFrances MC. Liver regeneration. Science 1997, *276*, 60-66.

Morello D, Fitzgerald MJ, C Babinet & N Fausto. C-myc, c-fos, and c-jun regulation in the regenerating livers of normal and H-2K/c-myc transgenic mice. Mol. Cell. Biol. 1990, 10, *6*, 3185-3193.

Miyake H, Kawabata G, Gotoh A, Fujisawa M, Okada H, Arakawa S, Kamidono S, Hara I. Comparison of surgical stress between laparoscopy and open surgery in the field of urology by measurement of humoral mediators. Int J Urol.2002, *6*, 329-333.

Narkowicz CK, JH Vial & PW McCartney. Hyperbaric oxygen therapy increases free radical levels in the blood of humans. Free Radic. Res. Commun. 1993, 19, *2*, 71-80.

Patockova J, Kriak M, P Marhol & E Tumova. Cerebrolysin inhibits lipid peroxidation induced by insulin hypoglycemia in the brain and heart of mice. Physiol. Res. 2003, *52*, 455-460.

Shen H, Yang C, J Liu & C Ong. Dual role of glutathione in selenite induced oxidative stress and apoptosis in human hepatome cells. Free. Radi. Biol. Med. 2000, *28*, 1115-1124.

Shimizu H, Miyazaki M, Yoshioka S, Ito H, Nakagawa K, Ambiru S. Changes in hepatic venous oxygen saturation related to the extent of regeneration after partial hepatectomy in rats. Am. J. Surg. 1999, *178*, 428-431.

Shimizu Y, Miyazaki M, Shimizu H, Ito H, Nakagawa K, Ambiru S, H Yoshidome & N Nakajima. Beneficial effects of arterialization

of the portal vein on extended hepatectomy. Br. J. Surg. 2000, *87*, 784-789.

Siddique M, Malik SA, Khan AS, G Nazir, & JA Chawla. Comparison of liver function tests after 2/3 hepatectomy and liver biopsy in experimental rats. J. Med. Sci. 2004, 4, *4*, 318-322.

Uwagawa T, Y Unemura & Y Yamazaki. Hyperbaric oxygenation after portal vein embolization for regeneration of the predicted remnant liver. J. Surg. Res. 2001, *100*, 63-68.

Waynforth HB & PA Flecknell. Hepatectomy in experimental and surgical technique in the rat. 2nd ed. Academic Press Ltd, London, 1992, pp 241-248.

Webber EM, FitzGerald MJ, Brown PI, MH Bartlett & N Fausto. Transforming growth factoralpha expression during liver regeneration after partial hepatectomy and toxic injury, and potential interactions between transforming growth factor-alpha and hepatocyte growth factor. Hepatology 1993, 18, *6*, 1422-1431.

Werner J, Fernandez-Del Castillo C, Rivera JA, Kollias N, Lewandrowski KB, DW Rattner & AL Warshaw. On the protective mechanisms of nitric oxide in acute pancreatitis. Gut 1998, *43*, 401-407.

Yasar M, Yildiz S, Mas R, Dundar K, Yildirim A, Korkmaz A, Akay C, Kaymakcioglu N, T Ozisik, & D Sen. The effect of hyperbaric oxygen treatment on oxidative stress in experimental acute necrotizing pancreatitis. Physiol. Res. 2003, 52, *1*, 111-116.

Yoshioka S, Miyazaki M, Shimizu H, Ito H, Nakagawa K, Ambiru S, N Nakajima & Y Fukuda. Hepatic venous hemoglobin oxygen saturation predicts regenerative status of remnant liver after partial hepatectomy in rats. Hepatology 1998, *27*, 1349-1353.