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Effect of hyperbaric oxygenation on the regeneration of the liver after partial hepatectomy in rats

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Abstract

The aim of the present study was to assess the influence of hyperbaric oxygenation (HBO) on rat liver regeneration before and after partial hepatectomy. Rats were sacrificed 54 h after 15% hepatectomy, liver and body weights were measured, and serum alanine transaminase (ALT) and aspartate transaminase (AST) activity and albumin levels were determined. The lipid peroxide level, as indicated by malondialdehyde production in the remnant liver was measured, and liver sections were analyzed by light microscopy. Five groups of 10 rats in each group were studied. The preHBO and pre-hyperbaric pressure (preHB) groups were treated before partial hepatectomy with 100% O_2 and 21% O_2, respectively, at 202,650 pascals, daily for 3 days (45 min/day). The control group was not treated before partial hepatectomy and recovered under normal ambient conditions after the procedure. Groups postHBO and postHB were treated after partial hepatectomy with HBO and HB, respectively, three times (45 min/day). The preHBO group presented a significant increase in the initiation of the regeneration process of the liver 54 h postoperatively. The liver/body weight ratio was 0.0618 ± 0.0084 in the preHBO compared to 0.0517 ± 0016 g/g in the control animals ($P = 0.016$). In addition, the preHBO group showed significant better liver function (evaluated by the lowest serum ALT and AST activities, $P = 0.002$ and $P = 0.008$, respectively) and showed a significant decrease in serum albumin levels compared to control ($P < 0.001$). Liver lipid peroxide concentration was lowest in the preHBO group ($P < 0.001$ *vs* control and postHBO group) and light microscopy revealed that the composition of liver lobules in the preHBO group was the closest to normal histological features. These results suggest that HBO pretreatment was beneficial for rat liver regeneration after partial hepatectomy.

Key words
- Partial hepatectomy
- Hyperbaric oxygenation
- Liver regeneration
- Alanine transaminase
- Aspartate transaminase

Introduction

Liver regeneration starts as a response to different types of liver tissue damage, including partial hepatectomy. After partial hepatectomy, liver tissue requires an increased amount of oxygen for mitochondrial oxidative phosphorylation to restore the hepatic energy charge (1). The increase in portal blood flow and oxygen supply produced by arterialization of the portal vein has beneficial effects on hepatic energy metabolism and liver regeneration, leading to improved survival after extended experimental hepatectomy (2). Hyperbaric oxygenation (HBO, 100% O_2 at 303,975 pascals for 1 h/day) after portal vein embolization improves the regeneration of the predicted remnant liver as indicated by hepatocyte growth factor, and percentage of proliferating cell nuclear antigen (3). This experimental model simulated conditions of liver regeneration similar to those after partial hepatectomy but partial hepatectomy was not performed (3). Rat hepatocytes are protected by hyperoxia (100% O_2 at 283,710 pascals for 1.5 h/day) against carbon tetrachloride-induced injury (4). The influence of HBO on recovery after partial hepatectomy has not been described.

Our hypothesis was that HBO would improve liver regeneration after partial hepatectomy. The aim of the investigation was to find which conditions of HBO treatment, before or after partial hepatectomy, would lead to improved liver regeneration. The influence of different HBO protocols on liver regeneration was estimated using several biochemical parameters, light microscopy, and the determination of the ratio liver mass to body mass which is a set point for growth regulation (5).

Material and Methods

Experiments were performed with male Wistar rats raised under controlled conditions (temperature of 22 ± 1ºC and a light schedule of 14-h light/10-h dark) at the Split University Animal Facility. Laboratory food and tap water were supplied *ad libitum*. Animals were bred and maintained according to the Guide for Care and Use of Laboratory Animals (NIH, 1996) and the protocol was approved by the Ethics Committee of the Split University Medical School.

One-month-old male Wistar rats were submitted to 15% partial hepatectomy because even small liver resections are sufficient to trigger regeneration in weanling rats (6). Rats weighing 73 to 102 g were submitted to partial hepatectomy by removing the left middle lobe. The operation lasted 15 min with diethylether as an anesthetic. All rats underwent partial hepatectomy and were then divided into 5 groups of 10 animals each. Fifty-four hours before partial hepatectomy, two groups were treated with HBO (100% O_2; preHBO group) and hyperbaric pressure (21% O_2; preHB group), respectively, daily for 3 days (45 min/day at 202,650 pascals). We also examined the influence of hyperbaric exposure (21% oxygen) to determine whether effect was due to increased oxygen level or to elevated pressure. The control group was not treated before partial hepatectomy and recovered under normal ambient conditions after the procedure.

Groups postHBO and postHB were treated after partial hepatectomy with HBO and HB, respectively, three times (45 min/day at 202,650 pascals). The animals were exposed to HBO and to HB in a Comex hyperbaric chamber (Comex, Marseilles, France). The oxygen and carbon dioxide concentrations in the chamber during exposure to hyperbaric oxygen were controlled by a Servomex 570A oxygen analyzer (Servomex, Houston, TX, USA) and by an infrared carbon dioxide gas analyzer (Infrared Industries Inc., Santa Barbara, CA, USA).

Blood samples were collected from the jugular vein into glass vacuum tubes without any anticoagulant, before sacrifice with prolonged anesthesia (diethylether). Serum alanine transaminase (ALT), aspartate trans-

aminase (AST) and albumin were determined with an automated BM/Hitachi 917 instrument (Boehringer Mannheim GmbH, Mannheim, Germany) using commercially available reagents from Roche Diagnostics GmbH (Mannheim, Germany). Animals were sacrificed 54 h after surgery because most of the changes in cell numbers during liver regeneration occur during this period (6), and liver and body weight were measured. Two sections were cut from the left and right lateral lobes of the liver of each animal, and fixed in 4% paraformaldehyde in 0.1 mM phosphate-buffered saline, pH 7.4, overnight and then dehydrated in increasing ethanol concentrations. The tissues were embedded in paraffin wax, cut into 5-µm sections and mounted on glass slides (7). Paraffin sections were stained with hematoxylin-eosin and analyzed with an Olympus BX 40 light microscope (Olympus, Tokyo, Japan). During histological examination special care was taken to compare sinusoid distribution and the presence of granulation and vacuoles in hepatocyte cytoplasm.

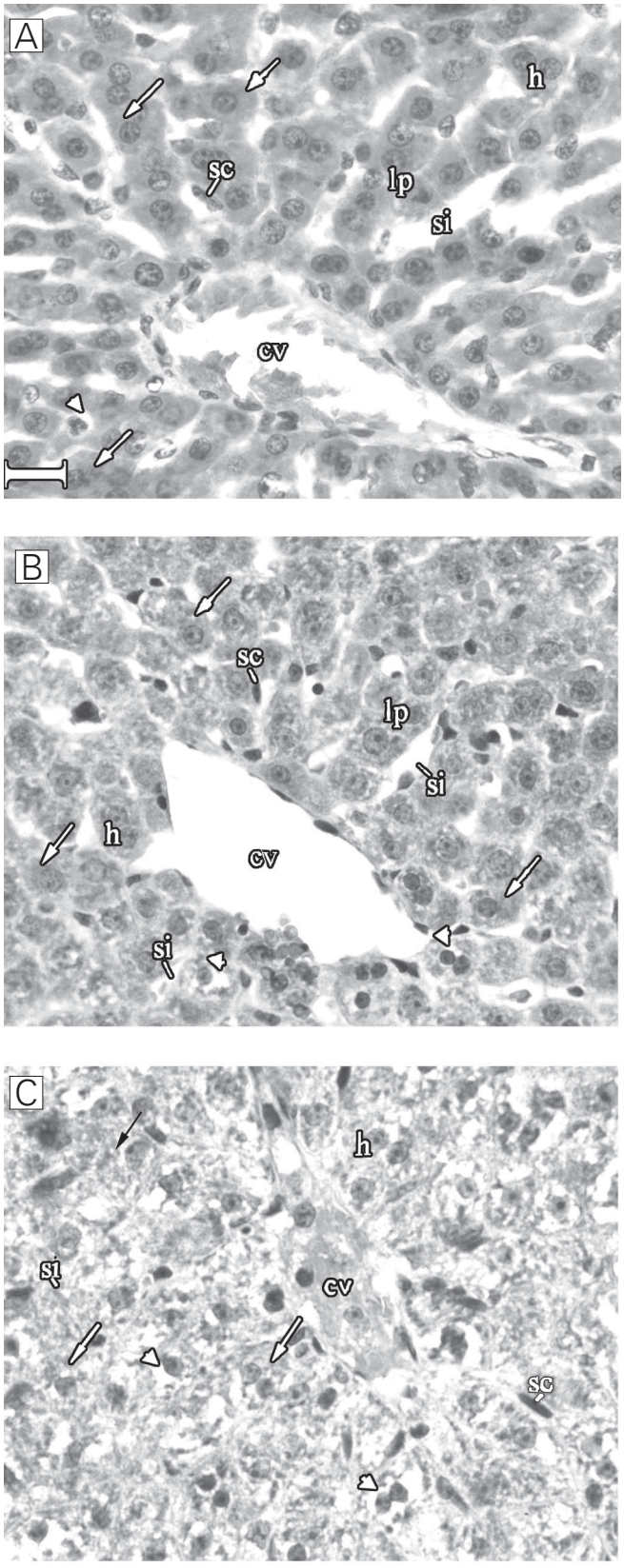
A standard procedure employing thiobarbituric acid (TBA) reaction under the conditions specified by Yagi (8) was used for the measurement of lipid peroxide levels in a liver tissue sample. The method is based on the reaction of lipid peroxides with TBA to yield a red pigment (9). Liver tissue samples were weighed and homogenates were prepared (10% w/v) using a glass homogenizer with ice-cold 0.15 M KCl. The homogenate (0.1 ml) was placed in a glass tube and mixed with 0.2 ml 8.1% sodium dodecyl sulfate, 1.5 ml acetic acid, pH 3.5, 1.5 ml 0.8% aqueous solution of TBA, and 0.7 ml distilled water. A marble was placed on each tube to limit evaporation and the samples were heated at 95°C for 60 min in a water bath, removed, and cooled. After cooling with tap water, 1.0 ml of distilled water and 5.0 ml of the mixture of *n*-butanol and pyridine (15:1, v/v) were added and shaken vigorously. After centrifugation at 4,000 rpm for 10 min, the *n*-butanol layer was taken for absorbance measurement at 532 nm. Malondialdehyde standards were prepared from 1,1,3,3-tetramethoxypropan. The lipid peroxide level in the liver tissue was calculated and reported in terms of malondialdehyde (nmol/1 g wet tissue). Experiments were performed in a model Specord 200 double-beam spectrophotometer (Analytik Jena GmbH, Jena, Germany).

Data are reported as means ± SEM for 10 rats in each group. The Kruskal-Wallis test followed by Dunn's test was used to assess the statistical significance of the results. Nonparametric tests were used due to small sample size

Table 1. Ratio of the wet weight of the remnant liver lobes to body weight, level of lipid peroxides in liver tissue expressed as malondialdehyde (MDA) equivalents, plasma albumin levels, and ALT and AST activity.

Variable	preHBO	preHB	Control	postHBO	postHB	P value
Liver wet weight/ body weight (g/g)	0.0618 ± 0.0084[a]	0.0545 ± 0.0021	0.0517 ± 0.0016[b]	0.056 ± 0.0014	0.0578 ± 0.0017	0.016
Lipid peroxides (nM/g MDA)	119.7 ± 4.9[a]	124.97 ± 6.3[a]	318.1 ± 24.6[b]	288.3 ± 12.4[b]	130.8 ± 7.1[a]	<0.001
Plasma albumin level (g/l)	20 ± 2.36[a]	29.7 ± 0.3	33.5 ± 0.7[b]	25.25 ± 0.37[a]	31.2 ± 0.59[b]	<0.001
ALT activity (U/l)	30.3 ± 5.09[a]	61.5 ± 8.6[b]	53.3 ± 4.43	67.8 ± 9.58[b]	60.2 ± 6.53[b]	0.002
AST activity (U/l)	101.1 ± 12.06[a]	161.2 ± 20.9	137.5 ± 6.26	218.38 ± 40.6[b]	171.9 ± 19.08[b]	0.008

All variables were measured 54 h after partial hepatectomy. Data are reported as means ± SEM for 10 rats in each group. preHBO = hyperbaric oxygenation (100% O_2) before partial hepatectomy; preHB = hyperbaric pressure (21% O_2) before partial hepatectomy; control = air at normal pressure before and after partial hepatectomy; postHBO = hyperbaric oxygenation (100% O_2) after partial hepatectomy; postHB = hyperbaric pressure (21% O_2) after partial hepatectomy; ALT = alanine transaminase; ASP = aspartate transaminase. Data followed by different letters were statistically different ($P < 0.05$ Kruskal-Wallis test followed by Dunn's test). Data without letters were not significantly different from any data.



(N = 10 each group). Mean values were considered to be significant at $P < 0.05$.

Results

At 54 h after partial hepatectomy, the mean ratio of the wet weight of the remnant lobes to body weight was highest in preHBO animals and lowest in control animals ($P < 0.050$, Table 1). The liver/body weight ratio was not significantly different among other groups examined. An inverse correlation was found between serum albumin levels and liver lipid peroxide levels. The highest lipid peroxide values were observed in the control group and the lowest, significantly different compared to control and postHBO groups, in the preHBO group (Table 1). The opposite was observed for serum albumin levels in preHBO and control groups. There was no significant difference in serum albumin levels between preHBO and postHBO groups. Serum enzymes (AST and ALT) were markedly lower in the preHBO than that in the postHBO and postHB groups (Table 1).

The light microscopy findings for preHBO-treated rats are shown in Figure 1A. Classical lobules consisting of hepatocyte plates and hepatic sinusoids radiated from the central vein in the liver. The liver plates, one or two hepatocyte layer thick, were bordered by sinusoids. Sinusoids and sinusoidal lining cells were distinctly seen among the liver plates. In addition, small and rare eosinophilic granules as well as small vacuoles were observed in the cytoplasm of hepatocytes (Figure 1A).

Figure 1. *A*, Liver tissue from a rat treated with hyperbaric oxygen before partial hepatectomy (preHBO group). *B*, Liver tissue from a control rat (recovered under normal ambient pressure after partial hepatectomy). *C*, Liver tissue from a rat treated with hyperbaric oxygen after partial hepatectomy (postHBO group). cv = central vein; h = hepatocyte; lp = liver plate; sc = sinusoidal lining cell; si = sinusoid; arrows = cytoplasmic granules; arrowheads = cytoplasmic vacuoles. Hematoxylin-eosin, 40X. Bar = 0.2 mm for all panels.

Figure 1B shows the liver of control group rats which recovered under normal ambient conditions after partial hepatectomy. Control livers were characterized by classical lobules composed of the plates of hepatocytes and hepatic sinusoids. The cytoplasm of the hepatocytes was moderately granulated, while some cells contained small clear vacuoles around centrally placed nuclei. No small fat vacuoles were seen. Sinusoids lined by sinusoidal lining cells, empty or sometimes filled with erythrocytes and bordered by the hepatocyte plates were easily recognized (Figure 1B).

The postHBO-treated liver, shown in Figure 1C, contained hepatocytes clustered around the central vein. The liver sinusoids, tortuous and empty or with erythrocyte-filled spaces, were scattered among the hepatocytes, although sinusoidal lining cells characterized by small, elongated and dark-stained nuclei were easily recognized. Individual hepatocytes were polygonal in shape, possessing one or two large, round and euchromatic (pale-stained) nuclei with dark nucleoli. Their cytoplasm contained numerous eosinophilic granules. In addition, clear vacuoles of various sizes surrounding central nuclei suggested a hydropic change occurring in the cells (Figure 1C).

Discussion

The present study demonstrated that hypobaric oxygen treatment before partial hepatectomy enhanced liver regeneration as indicated by the higher ratio of the wet weight of the remnant liver lobes to the body weight compared to the other groups examined. This protective effect of HBO pretreatment was accompanied by lower tissue lipid peroxidation and lower levels of serum aminotransferases in the same group. There is evidence suggesting that lipid peroxidation inversely correlates with the rate of mitosis (10). Liver regeneration occurs in two phases, priming and cell cycle progression. Priming is initiated by cytokines such as tumor necrosis factor (TNF) and interleukin-6 (11). Reactive oxygen species and glutathione content can determine whether the TNF effect on hepatocytes is proliferative or apoptotic (5). Repeated HBO inhalation can alter enzymatic antioxidant activity. Gregorevic et al. (12) detected a 241% increase in superoxide dismutase activity in rat skeletal muscle after repeated HBO. It is tempting to speculate that preconditioning with HBO before partial hepatectomy improves antioxidant defensive mechanisms. Presumably TNF exerted its proliferative signalling effect due to the recovery of preHBO-treated rats under normal ambiental conditions and due to the decreased oxygen radical formation (indirectly indicated in our experiment by lipid peroxide concentration).

Liver regeneration is coupled with transcriptional rearrangement of numerous genes (13). Using a liver chip, 116 different RNAs were identified during regeneration. Twelve to 13 h after partial hepatectomy, the highest up-regulation (a 12-fold change) was detected for RNA coding a positive acute phase response protein, alpha 1-acid glycoprotein, and RNA encoding albumin reaches its nadir at 24 h after surgery (14). Wenger et al. (15) described oxygen-regulated acute phase gene expression in a hepatoma cell line. Our finding of significantly lower serum albumin levels in the preHBO group agrees with results observed by others and could mean that transition phase of liver regeneration, characterized by a switch from albumin gene suppression to activation, is reached earlier in this group than in other groups examined (16-18).

HBO treatment after partial hepatectomy did not interfere with liver regeneration, as indicated by wet weight and serum albumin levels. In contrast, there was an increase in serum aminotransferase and in free radical production, indicating greater liver injury. The beneficial effect of HBO largely depends on the duration and timing of treat-

ment in relation to ischemia-reperfusion injury (before, during, after, or delayed) (19). According to the observation that delayed treatment of rats with HBO after carbon tetrachloride-induced injury was less effective in preventing necrosis than immediate treatment (4), we found that postHBO treatment could be toxic compared to the beneficial effect of preHBO treatment. Partial hepatectomy *per se* is related to the formation of free radicals that can induce cell damage dependent on tissue oxygen concentration and on the activity of the enzymatic and nonenzymatic systems involved in tissue protection. Several investigators have suggested an increased production of oxygen radicals measured by malonyldialdehyde in liver mitochondria following partial hepatectomy, which can alter the function of the enzymes involved in oxidative phosphorylation (20-23). Hepatectomy-induced lipoperoxidation in resected liver may inhibit activation of mitochondrial respiration and hepatic regeneration (24). HBO applied after partial hepatectomy could increase lipid peroxide content in already injured liver. Since that reactive oxygen species content can determine whether the TNF effect on hepatocytes will be proliferative or apoptotic (5) and based on our finding of high lipid peroxide concentrations in the livers of the postHBO group, we speculate that the effect of the TNF signaling pathway was presumably apoptotic in postHBO group. Conversely, lipid peroxide content was low in the preHBO group, and therefore the influence of TNF may have been proliferative. The mechanism proposed is hypothetical and requires additional experiments to elucidate the modulation of liver regeneration by HBO.

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