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The Effects of Hyperbaric Oxygen Treatment on Oxidant and Antioxidants Levels during Liver Regeneration in Rats

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[1]Department of Biochemsitry, [2]Department of General Surgery, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, [3]Department of Underwater and Hyperbaric Medicine, Istanbul Medical Faculty, Istanbul University, [4]Department of Physiology, [5]Department of Pathology, Cerrahpasa Medical Faculty, [6]Department of Public Health, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey

Özden, T.A., Uzun, H., Bohloli, M., Toklu, A.S., Paksoy, M., Simsek G., Durak, H., Issever, H. and İpek, T. *The Effects of Hyperbaric Oxygen Treatment on Oxidant and Antioxidants Levels during Liver Regeneration in Rats.* Tohoku J. Exp. Med., 2004, **203** (4), 253-265 —— The effects of hyperbaric oxygen (HBO) therapy on oxidant/antioxidant metabolism are controversial and its effects on hepatic regeneration are not known. In this study, we investigated a possible beneficial effect of HBO therapy on oxidant and antioxidants levels during liver regeneration. To conduct this study, seventy percent hepatectomy was performed on forty-eight Spraggue-Dawley rats and the rats were divided into two equal groups: HBO-treated group and untreated group (non-HBO group). We determined the levels of malondialdehyde (MDA), an oxidative stress marker, and the levels of antioxidant enzymes/reagents, including glutathione (GSH), superoxide dismutase (SOD) activitiy, copper (Cu) and zinc (Zn), in the remnant liver samples. We also measured mitotic index (MI) and proliferating cell nuclear antigen (PCNA) levels to assess the degree of liver regeneration. HBO treatment significantly decreased MDA levels, whereas it increased SOD activity, GSH and Zn levels. In contrast, Cu levels were lower in the HBO-treated livers than the levels in the untreated remnant livers. The effect of HBO treatment may be mediated by the suppression of certain enzymes that are responsible for lipid peroxidation. In addition, HBO treatment may induce the production of antioxidant enzymes/reagents by remnant liver tissues. The HBO-treated rats maintained their body weights but the untreated rats lost body weights. HBO treatment also increased MI and PCNA levels, indicating HBO treatment enhances liver regeneration. These

results indicate that HBO treatment has beneficial effects on liver regeneration by decreasing MDA and by increasing antioxidant activities. We therefore suggest that HBO therapy may be useful after liver resection. ——— hyperbaric oxygen treatment; liver regeneration; oxidant; antioxidant; trace elements
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Hyperbaric oxygen therapy (HBO) is a mode of medical treatment in which the patient is entirely enclosed in a pressure chamber breathing 100% oxygen at a pressure greater than one atmosphere. HBO is used for the treatment of various inflammatory diseases and ischemic conditions (Cohn 1986; Gabb et al. 1987; Grim et al. 1989). Although HBO therapy is a useful method for treating various clinical conditions such as wound healing, carbon monoxide poisoning, and acute necrotizing infections (Bakker 1992; Fitzpatrick 1994), it can also cause some side effects including barotraumas (sinus and middle air barotrauma, tympanic membrane rupture, pneumothorax, air embolism due to pulmonary barotrauma), oxygen toxicity (Central nervous toxic reactions, pulmonary toxic reactions), reversible visual change and claustrophobia (Gabb et al. 1987; Grim et al. 1989; Bakker 1992; Hampson 1999; Chavko 2001). The role of HBO therapy in free radical-mediated tissue injury and oxidant/antioxidant metabolism is controversial and its effect on hepatic regeneration are not known (Monstrey et al. 1997). Repeated exposure to HBO or hyperoxia may be expected to lead to an increase in the formation of hydrogen peroxide (H_2O_2) (Harrison et al. 1976), whereas it may also induce the production of antioxidant enzymes/reagents by tissues (Haugaard 1968; Harabin et al. 1990).

Normal cellular metabolism involves the production of reactive oxygen species (ROS) (McCord et al. 1993). Low levels of ROS are vital for many cell signaling events and essential for proper cell functioning (Harabin et al. 1990; Gregorevic et al. 2001; Fattman et al. 2003), but excessive in vivo generation of ROS can adversely affect cell functioning (Gregorevic et al. 2001). The consequence of ischemic-reperfusion (I/R) during liver transplantation, hepatic resectional surgery and hemorrhagic shock appears to be an acute inflammatory response followed by dramatic cellular damage and organ dysfunction (Bilzer et al. 2000). ROS, likely to afflict direct tissue damage, are destructive products generated during I/R (Bilzer et al. 2000; Khandoga et al. 2003). Superoxide (O_2^-) produced from a one-electron reduction of oxygen can be modified to H_2O_2, undergoing either spontaneous or enzyme-catalyzed dismutation. These ROS can react with a variety of cellular macromolecules such as lipids, proteins, DNA, and $(Fe-S)_4$ centers, leading to the disruption of cell membranes, inappropriate activation or inactivation of enzymes, and genetic mutations (Cheeseman et al. 1992; Gonzales 1992). End products formed during the lipid peroxidation process including MDA are very reactive and capable of cross-linking of membrane proteins containing amino groups (Kehrer 1993).

Organisms have developed a variety of antioxidant defenses to protect against oxidative damage (Fattman et al. 2003). Antioxidants and antioxidant enzymes in an organism may operate within the context of an integral system. Components of the systems may work in concert or synergistically scavenge reactive oxygen species, preventing oxidative damage (Shang et al. 2002; Fattman et al. 2003). GSH is an important peptide with multiple functions ranging from antioxidant defense to modulation cell proliferation (Lu 1999) and it serves several vital functions such as preventing the oxidation of –SH groups and modulating critical cellular processes as DNA synthesis. (Meister 1988; Suthanthiran et al. 1990; DeLeve et al. 1991; Hutter et al. 1997). As an antioxidant enzyme, superoxide dismutase (SOD EC 1.15.1.1) removes ROS from the cel-

lular environment by catalyzing the dismutation of the two superoxide radicals to hydrogen peroxide and oxygen (McCord 1969; Marklund 1985; McCord 1993). It exists as a 32-kDa homodimer (Marklund 1982) and is present in the cytoplasm and nucleus of every cell type examined, in which it acts as a bulk scavenger of superoxide (Crapo et al. 1992; Oury et al. 1995). This protective enzyme requires copper (Cu) and zinc (Zn) for their optimal function and a deficiency in any of these trace elements may cause a decrease in the activity of the enzyme. Cu plays a major role in redox-reactions, participating via the Haber-Weiss reaction in the generation of free oxygen radicals, which promotes cellular instability. Zn, has also been shown to play a vital role in protecting the tissues from the damage caused by free radicals (Willson 1987; Willson 1989; Bremner 1995).

There is no clinical method for serial monitoring of hepatic regeneration after hepatoctemy in vivo, however mitotic index (MI), some specific enzyme activity and proliferating cell nuclear antigen (PCNA), the labeling index of liver have been used as experimental parameters to monitor liver regeneration (Shimizu et al. 1999). The present study examined the effects of HBO treatment on the levels of MDA, GSH, Cu, Zn, SOD, Cu, and Zn during liver regeneration in rats. MI and PCNA were used to monitor the liver regeneration.

MATERIALS AND METHODS

Animals

Forty-eight Spraggue-Dawley rats weighing between 210 and 380 g were obtained from Istanbul University, Cerrahpasa Medical Faculty, Animal Breeding and Research Laboratory. All experiments were done in according to the standards in "The UFAW Handbook on the Care and Management of Laboratory Animals" (Poole 1999). The rats were kept in standard cages, with 8 rats in each cage at room temperature. The animals were fed with standard rat chow and tap water *ad libitum* both pre- and postoperatively. Seventy percent hepatectomy was performed on all rats and the rats were divided into two equal groups: HBO-treated and non-treated groups. The rats in HBO- treated group received HBO therapy after hepatectomy, whereas the rats in non-treated group did not. The rats in both groups were sacrificed on the second day, the fourth day, and the seventh day after hepatectomy. The control liver specimens were prepared from sixteen rats during hepatectomy. The body weights of the rats were recorded after the hepatectomy to assess the beneficial effects of HBO treatment. The operations were conducted between 9 and 12 o'clock a.m. in order to standardize the effects of diurnal changes. The rats were given ether anesthesia in a closed jar. Their abdominal walls were cleansed with polyvinylpyrolidone iode solution after shaving. Median incision was made. The left and median lobes of the liver were exposed to standard 70% hepatectomy, applying the technique defined by Higgins and Anderson (Higgins et al. 1931). Initially the peduncle of the left lobe was resected with 4/0 silk. 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 suture as a single layer. All of the operations were performed in a clean, but not sterile condition. Neither operative nor postoperative loss occurred in the groups. Thoracotomy and laparotomy were performed at the time of sacrifice. Liver tissue samples of the rats were taken for biochemical analyses in a disposable cup. After collecting the liver samples, they 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 fixated in 10% formaldehyde solution.

HBO treatment

HBO treatment commenced about 3 hours after the operation and lasted for seven days. The HBO treatment protocol was set as four times a day in the first two days, three times a day on the third and fourth days and twice a day during the last three days. The treatments were con-

ducted in a small research chamber (0.28 m^3) at the Department of Underwater and Hyperbaric Medicine, Istanbul Faculty of Medicine. The chamber was flushed with oxygen for 10 minutes to vent the air inside before the compression, so that the animals could be pressurized with 100% oxygen. The HBO treatments were 80 minutes at 2, 5 atmospheres absolute (ATA) including 10 minutes compression and 10 minutes decompression time.

Sample preparation

Preparation of tissue specimens for MDA, GSH, and SOD. The liver tissue samples were weighed and minced and then homogenized (10% w/v) using 66-mmol/l-phosphate buffer (pH: 7, 0). A part of the homogenate was used for the estimating MDA levels and the remainder was centrifuged at 1 000×*g* for 15 minutes at 4°C. The obtained supernatant was further centrifuged at 12 000×*g* for 20 minutes at 4°C to obtain the postmitochondrial supernatant to be used for estimating GSH levels and SOD activity (Lowry et al. 1951; Buege et al. 1978; Beutler et al. 1983; Sun et al. 1988; Neve et al. 1995).

Preparation of tissue specimens for Cu and Zn. Specimens were obtained with special care to avoid contamination. Plastic instruments were used. Tissue specimens were weighed and then dried in an oven at 100°±2°C to a constant weight for 36 hours. The cooled and dried tissue samples were extracted three times using 5 ml aliquots of a mixture of equal volumes of diethyl ether and petroleum ether. Then tissue samples were again dried in an oven at 100°C±2°C to a constant weight. 1 ml of 30% H_2O_2 was added to approximately 50 mg samples of dry defatted tissue and mixed gently by swirling. After placing the tissue samples in an oven at 65°-70°C for drying, 1 ml of 30% H_2O was added to dry tissues for digestion three times. When digestion was complete, dried digest was dissolved in 2.0 ml of 0.5 M nitric acid and diluted to 10 ml using bidistilled water (Alcock 1987; Chou 1987).

Biochemical assays

Liver MDA levels were measured using thiobarbitıric acid method described by Buege and Aust (1987) and MDA results were expressed as nmol/mg protein. SOD activity was calculated by using the nitro blue tetrazolium assay (Sun et al. 1988). The protein contents used in determining the SOD, GSH, and MDA levels were measured by the method of Lowry et al. (1951) using bovine albumin as a standard. SOD results were expressed as U/mg protein and GSH results as μg/mg protein. All chemicals were obtained from Sigma Chemical Co. (Sigma-Aldrich Chemia Gmbh, Steinhei, Germany).

Tissue Cu and Zn measurements. Standard solutions for Zn and Cu were prepared with 1% nitric acid (Merck, Darmstadt, F, R, Germany) from 10 ppm stock standard (Fisher Scientific Comp., NJ, USA). Tissue Zn and Cu levels were measured using flameless atomic absorption spectrophotometer (Varian spectra AA-200 [Varian Australia Pty Ltd.]), and a graphite furnace (GTA-100 [Varian Australia Pty Ltd.]). Cu and Zn results were expressed as μg/g dry liver tissue (Alcock 1987; Chou 1987).

Preparation of tissue specimens for Mitotic Index and PCNA values. In an attempt to determine MI and PCNA values, a portion part of these liver samples was fixated in 10% formaldehyde solution. The tissue samples were embedded in paraffin blocks, cut with microtome in 3-5 micron thickness, taken on the slides, numbered, and stained with hemotoxylin-eosin. The stained samples were examined under a light microscope (Olympus BX50, Tokyo) with 400× magnification. Mitotic index for each sample was calculated by means of the number of mitotic figures in 10 fields.

To determine PCNA values (Hall et al. 1990), other tissue sections on the slides were undergone antigen retrieval for 20 minutes in 800 W microwave oven in citrate buffer with pH 6 after deparaffinization. Predilution was done with PCNA antibodies (Dako, Glostrup, Denmark) (Labelled avidin-biotin complex technique), with

chromogen (3-amino-9-ethylcarozole) and haematoxylin as contrast stain. The slides were examined under an Olympus BX50 light microscope. The percentage of the cells stained with immunohistochemical PCNA staining were calculated by counting the stained cells in 10 different fields for each individual rat under 400× magnification.

Statistical analysis

After the one–way ANOVA test, Tukey test was used for multiple comparisons and a paired *t*-test was used in order to find out whether HBO treatment had any effects on the weight of the rats. The level of $p<0.05$ was assumed to be statistically significant.

RESULTS

The changes in the levels of MDA, GSH, SOD, Cu and Zn in the remnant livers of the HBO-treated and untreated rats are summarized together with the values in the control livers (Table 1). There were significant differences in the MDA values among all remnant liver samples after hepatectomy ($F_{6;63}$=189.5, $p<0.001$). The MDA levels were significantly decreased ($p<0.05$) by 4 days after treatment with HBO (Table 1). The MDA level was the lowest in the control livers when compared to the MDA values in the remnant livers of HBO-treated and untreated rats ($p<0.05$).

There were significant differences in the GSH levels among all of the groups ($F_{6;63}$=29.63, $p<0.001$). The GSH levels were significantly higher in the remnant livers of the rats treated with HBO for 4 or 7 days than those of the rats treated with HBO for 2 days ($p<0.05$). The GSH levels significantly decreased in untreated livers by 4 days after hepatectomy ($p<0.05$). The untreated remnant livers showed the lowest values on 4 and 7 days after hepatectomy, which were even lower than the values in the control liver (Table 1).

We also detected a significant differences in the SOD levels among all of the groups

TABLE 1. *Oxidant and anti-oxidant parameters levels in the remnant liver tissues of HBO-treated (HBO) and untreated (non-HBO) rats during liver regeneration. For comparison, the values in control liver tissues are shown (Control). Each value represents the mean±S.D.*

Groups	HBO 2 days (*n*: 8)	HBO 4 days (*n*: 8)	HBO 7 days (*n*: 8)	non-HBO 2 days (*n*: 8)	non-HBO 4 days (*n*: 8)	non-HBO 7 days (*n*: 8)	Control Liver tissues (*n*: 16)	F
MDA (nmol/mg protein)	1.24±0.2	0.66±0.1	0.61±0.04	1.28±0.1	1.34±0.1	1.36±0.1	0.5±0.1	189.5*
GSH (μg/mg protein)	2.8±0.2	3.9±0.3	4.0±0.9	2.7±0.50	2.2±0.3	2.2±0.4	3.0±0.35	29.63*
Cu, ZnSOD (U/mg protein)	0.82±0.06	0.92±0.12	0.91±0.12	0.74±0.18	0.76±0.24	0.58±0.2	0.58±0.1	8.33*
Cu (μg/g dry tissue)	13.0±3.3	14.3±2.0	21.5±8.8	44.0±10.3	31.12±8.9	32.0±5.2	31±6.4	21.67*
Zn (μg/g dry tissue)	110.2±25.0	137.8±36.2	139.6±34.2	105.0±26.5	91.9±28.1	82.7±17.3	72.2±10.0	13.81*

F: One Way ANOVA test, degrees of freedom (df): 6.63
* Statistically significant for each parameter among all groups ($p<0.001$).

($F_{6;63}$=8.33, $p<0.001$). The SOD levels were significantly increased in the remnant livers of the HBO-treated rats comparing to those in the untreated remnant livers and the control liver ($p<0.05$) (Table 1).

The changes in Cu levels among all of the groups were statistically significant ($F_{6;63}$=21.7, $p<0.001$). The increase in the Cu levels was statistically significant by 4 days after treatment with HBO ($p<0.05$). However the Cu levels in untreated remnant livers and control livers were higher than the HBO-treated livers (Table 1).

There was a statistical significance in the level of Zn among the groups ($F_{6;63}$=13.81, $p<0.001$). The Zn levels were increased in the liver by 4 days after treatment with HBO ($p<0.05$), whereas the levels were significantly decreased in untreated livers by 4 days after hepatectomy ($p<0.05$) (Table 1).

The MI and PCNA values in the HBO-treated and untreated rat livers are summarized in Table 2. There was also a significant difference in the MI values among all of the groups. ($F_{5;47}$=21.03, $p<0.001$). The MI values were significantly higher in the liver samples of the HBO-treated rats than those of the untreated rats (Table 2, Fig. 1).

The differences in the PCNA levels among the groups were also statistically significant ($F_{5;47}$=98.63, $p<0.001$). The PCNA levels were significantly higher in the remnant livers after treatment with HBO for 2 or 4 days compared to those of untreated rats (Table 2). The PCNA values reached about 65% on 2 days and 70% on

TABLE 2. *Mitotoic index (MI) and proliferating cell nuclear antigen (PCNA) values in the remnant liver tissues of HBO-treated (HBO) and untreated (non-HBO) rats during liver regeneration. Each value represents the mean±S.D.*

Groups	HBO			non-HBO			F
	2 days (*n*: 8)	4 days (*n*: 8)	7 days (*n*: 8)	2 days (*n*: 8)	4 days (*n*: 8)	7 days (*n*: 8)	
MI	4.83±2.24	3.8±1.77	0.61±0.04	0.69±0.30	0.22±0.10	0.34±0.12	21.03[*]
PCNA (%)	63.78+11.59	69.30+19.09	4.0±0.9	2.52+1.53	1.41+0.30	7.11+1.69	98.63[*]

F: One Way ANOVA test, degrees of freedom (df): 5.47

[*] Statistically significant for each parameter among all groups ($p<0.001$)
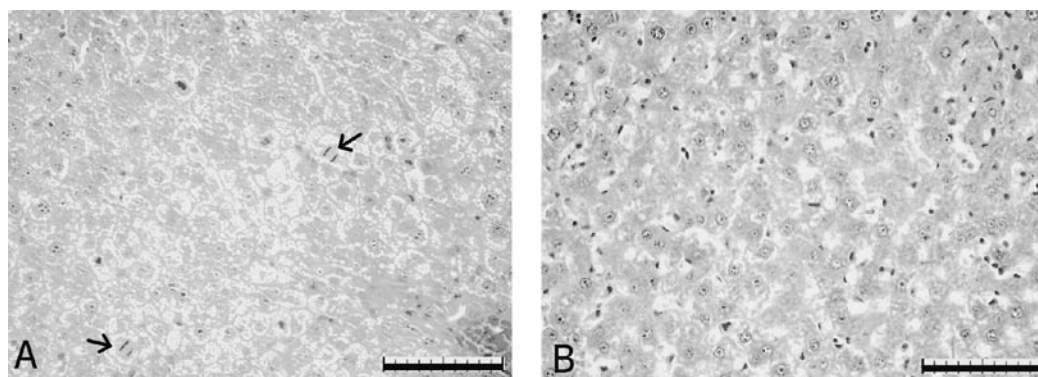

Fig. 1. Mitotic index in the remnant rat liver after hepatectomy. A: High mitotic index in the liver tissues of rats treated with HBO for 2 days. Arrows show mitosis. B: Low mitotic index in the liver tissues of untreated rats on 2 days after hepatectomy. Specimens were stained by hematoxylin-eosin.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

TABLE 3. *The weights of the rats after hepatectomy and before sacrifice. Each value represents the mean±* s.d.

Groups	HBO			non-HBO		
	2 days (*n*: 8)	4 days (*n*: 8)	7 days (*n*: 8)	2 days (*n*: 8)	4 days (*n*: 8)	7 days (*n*: 8)
Weight after hepatectomy (g)	284.87±31.97	299.5±44.5	274.62±41.02	308.5±32.85	295±31.61	294.50±11.55
Weight before sacrifice (g)	288.25±32	299.62±43.96	281.87±40.82	304.25±32.15	286.37±31.36	288.75±13.46
Difference between the weights	3.38±11.25	0.12±3.25	7.25±14.75	−4.25±12.25	−8.63±7.50	−5.75±6.10
t	0.84	0.10	1.38	0.97	3.24[*]	2.66[*]

t, *t*-values according to paired-*t* test.
[*] Statistically significan decrease in weight of the untreated rats ($p<0.05$).

4 days after HBO treatment, but then decreased to 4% by 7 days. In contrast, the PCNA level was highest (about 7%) in the remnant liver tissues of the untreated rats on 7 days after hepatectomy.

The body weights of the HBO-treated rats did not change, whereas the untreated rats significantly decreased their body weights by 4 and 7 days after hepatectomy (Table 3).

DISCUSSION

In some clinical settings, such as liver transplantation, hepatic resectional surgery, and hemorrhagic shock, as an outgrowth of I/R injury in liver, microcirculatory derangement, energy depletion, production of ROS and lipid peroxidation occur (Chen et al. 1998; Bilzer et al. 2000). ROS cause inflammation and cell death through modulation of signal transduction pathways by affecting redox-sensitivity enzyme and transcription factors, by supporting protease activity, and by stimulating the expression of inflammatory mediators and adhesion molecules (Bilzer et al. 2000; Jacschke 2000).

In the recovery of hepatocellular function after severe traumas, free oxygen radicals should be kept in the normal ranges (Jarrar et al. 2000).

In addition to controversial data about the effects of HBO treatment on free oxygen radicals,

its effects on hepatic regeneration are not yet known (Narkowicz et al. 1993; Monstrey 1997). There is no established usage of HBO treatment in liver diseases (Bhattarcharya et al. 1996). Although some free radicals are produced during HBO treatment, the procedure is considered to be safe since the activity of some of the free radical scavenger is increased. On the other hand, for the safety reason, the HBO treatment pressure never exceeds 3 atm and it usually doesn't last longer than 90 minutes. If these safety guidelines are not followed, free radicals may accumulate and may cause oxygen toxicity in the central nervous system or in the lungs. (McCord et al. 1969; Gregory et al. 1973; Kimball et al. 1976). Bhattacharya et al. (1996) found that HBO treatment decreased complication rates in patients with chronic viral hepatitis and had positive effects on liver functions. Kudchodkar et al. (2000) showed that HBO treatment resulted in a marked reduction in TBARS (Thiobarbituric Acid Reactive Substances) formation in plasma and various tissues according to the non-HBO treatment. HBO treatment prevented the increase in liver TBARS seen in rabbits receiving cholesterol-rich diet. Reduced oxidation of tissue and plasma lipids may well be a result of induced antioxidant activity. Chen et al. (1988) showed that MDA level is

reduced, ATP is increased by HBO treatment on hepatic I/R injury. The protective effect of HBO treatment may be mediated by the suppression of specific enzymes that are responsible for lipid peroxidation (Kudchodar et al. 2000). According to Andiran et al. (2003), MDA levels of regenerated cirrhotic livers are significantly higher than those of cirrhotic liver. However, MDA levels of normal and regenerated normal livers did not differ significantly. In our study, the MDA levels in the untreated remnant liver tissues were increased and reached the highest values by 4 or 7 days after hepatectomy, whereas the GSH levels were decreased to the minimum level by 4 days after hepatectomy. These findings suggest that hepatic resectional surgery caused cellular damage. Bilzer et al. (2000) have found that hepatic resectional surgery causes an acute inflammatory response, followed by dramatic cellular damage and organ dysfunction. In our study, MDA levels in the remnant liver tissues of the HBO-treated rats were significantly decreased by 4 days after hepatectomy comparing to those of the untreated rats (Table 1). However, the MDA levels in the control liver tissues were the lowest. These results suggest that HBO treatment may decrease liver damage after hepatic resection. Our findings about MDA levels are in accordance with the results of the previous studies (Chen et al. 1998; Kudchodkar et al. 2000; Shang et al. 2002).

Notably, the GSH levels were consistently higher in the remnant liver tissues of the HBO-treated rats than those of the untreated rats and the levels of the control liver tissues (Table 1). The increase in the levels of GSH may represent an adaptive response to oxidative stress and similar adaptive induction of antioxidants or endogenous antioxidant enzymes has been observed in various animal models (Kimball et al. 1976; Thornalley et al. 1985; Purucker et al. 1992; Shang et al. 2002).

Tsai et al. (1992) reported that the changes in the mitocondrial respiration in the early phase of resection were due to the tissue injury resulting from the increase in free radicals. These free radicals are removed from the tissue by superoxide dismutase to prevent the tissue injury (Shimizu et al. 1999). In this study, liver SOD activity was found to be higher in the HBO groups than the activity in the non-HBO groups and the control group (Table 1). The SOD activity increased by the effect of HBO treatment was thought to prevent hepatic mitocondrial oxidation by the free radicals and to have beneficial effects on hepatic regeneration.

Zn and Cu are mutual antagonist trace elements and there is a competitive biological interaction between them (Bremner et al. 1976; Bremner et al. 1995). Although changes in the activity of Cu enzymes may occur in animals or in cells exposed to high levels of Zn, it is not certain that Cu in the enzymes is displaced by Zn, rather it seems that the amount of Cu has been reduced. (Bremner et al. 1995). Abul et al. (2002) have indicated that the changes of antioxidant levels are correlated with the changes of trace element levels, and suggested that antioxidants alone or with trace elements may have beneficial effects in treating liver cirrhosis. We were unable to find any data about the effects of HBO treatment on Cu and Zn levels during liver regeneration. In our study, Zn levels and SOD activity are higher in the remnant liver tissues of the HBO-treated rats than those of the untreated rats and the levels of the control liver tissues. Notably, Cu levels were also increased in the HBO-treated rats, but these levels were significantly lower than the Cu levels of untreated rats (Table 1).

Some authors showed that the increase in the hepatic venous hemoglobin oxygen content is necessary for the mitochondrial oxidative phosphorilation in the remaining liver after resection and provides the energy for hepatic regeneration (Yoshioka et al. 1998; Shimizu et al. 1999). HBO treatment increases the amount of dissolved oxygen in plasma. The remnant liver requires an increased amount of oxygen in relation to the extent of regeneration and optimal oxygen saturation is required during the regenerative period (Pruthi et al. 1993; Shimizu 1999). The

increased tissue oxygen gradient provided by HBO treatment enhances fibroblastic proliferation, collagen synthesis, angiogenesis and neovascularization, oxidative killing of the leukocytes (Cohn 1986). Furthermore, HBO treatment decreases adherent leukocyte count and the levels of the MDA-an oxidative stress marker, whereas it increases flow velocity in postsinosoidal venules (Chen et al. 1998). Some authors showed that HBO treatment decreased liver necrosis induced by carbon tetrachloride (Burk et al. 1986; Marzella et al. 1986).

We investigated a possible beneficial effect of HBO treatment on oxidant and antioxidant levels during liver regeneration. GSH, SOD and Zn levels in the remnant liver tissues were higher in the HBO groups than in the untreated groups. The MDA and Cu levels in the liver tissues were lower in the HBO groups than the levels in the untreated groups. Oxidative stress is known to play an important role in the pathogenesis of liver injury (Chen et al. 1998; Bilzer et al. 2000; Jacschke 2000; Kaplowitz 2000). Low levels of ROS and high contents of GSH inhibit lipid peroxidation and liver necrosis. Conversely, high levels of ROS and low levels of GSH can be deleterious by enhancing lipid peroxidation and apoptosis (Peterhans 1997; Jones et al. 1998; Vos et al. 1999; Rolla et al. 2000; Kaplowitz 2000). By inducing GSH depletion, ROS induce oxidative stress and reduce the antioxidant capability of other antioxidants (Huang et al. 1998; Nishikawa et al. 1998; Hall 1999). Depletion in GSH may also be a consequence of liver damage (Loguercio et al. 2002). It has been suggested that the measurement of GSH, SOD and lipid peroxidation may represent a useful marker in monitoring the progression of liver damage and the response to the therapy in patients suffering from liver disease (Loguercio et al. 1997; Sakaguchi et al. 2000; Shen et al. 2000; Atkinson et al. 2001; Loguercio et al. 2001). HBO treatment also caused a significant increase in Zn levels. Zn, as an antioxidant trace element, protects liver microsomes against lipid peroxidation damage (Chvapil

et al. 1973). It has been demonstrated that there were relations between high lipid peroxidation and abnormal membrane morphology in tissues from Zn deficient animals (Cherr et al. 1991).

MI and PCNA are useful markers to monitor the progression of liver regeneration after hepatoctemy. MI is increased only during regeneration; hepatocytes are inactive in the normal subjects (Brues et al. 1936; Hays et al. 1974). In the present study, MI values in the HBO groups were significantly increased in comparison to the non-HBO groups (Table 2, Fig. 1). PCNA value is also increased in the regenerating cells (Hays et al. 1974; Hall et al. 1990; Waseem et al. 1990) and it is a good parameter to monitor regenerating liver tissue (Hall et al. 1990; Shimizu et al. 1999). Eguchis et al. (1999) showed PCNA values as 40% in transplanted patients. In our study, PCNA values reached about 65% in the remnant liver tissues of HBO-treated rats (Table 2).

It has become clear that free radical chemistry plays an important role in the initiation of several disorders (Cross 1987). Therefore, the development of substances that have free radical scavenging properties or capability of stimulating antioxidant enzyme activities (SOD, GSH) represents an important field in the therapeutic researches (Louajri et al. 2001). In the present study, the decrease in the levels of MDA, oxidative stress marker, and the increase in the levels of GSH and SOD, which have free radical scavenging properties, showed that HBO treatment diminished oxidative stress by increasing antioxidant enzymes. Likewise, HBO treatment enhanced the liver regeneration, as judged by the MI and PCNA values. The body weights of the HBO-treated rats did not change while the untreated rats lost weight. These findings suggest that HBO treatment may have a therapeutic effect on liver regeneration after resection. We propose that HBO treatment may induce antioxidant mechanism and decrease the oxidative stress. Thus, HBO therapy may have beneficial effects on liver regeneration.

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