Effect of Hyperbaric Oxygen on Liver Regeneration in a Rat Model

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ABSTRACT

Hyperbaric oxygen therapy is a treatment that has been gradually implemented for the treatment of several pathologic conditions. The present study evaluated the effect of hyperbaric oxygen therapy for hepatic regeneration and its relationship to mitochondrial function. Male Wistar rats underwent partial hepatectomy (70%) and subsequently underwent two sessions of hyperbaric oxygen (90 minutes each, at a pressure of 2 ATA). The animals were sacrificed at 24 and 48 hours after surgery. Hepatic regeneration was evaluated by the dry weight of the remaining liver, the hepatic regeneration rate, the hepatic DNA content, and the hepatocyte proliferation rate using the "proliferating cell nuclear antigen" (PCNA) content. Function of the mitochondria was evaluated by its oxygen consumption during respiratory states 3 and 4, its respiratory control ratio (RCR), its membrane potential, as well as its osmotic swelling. We also measured serum levels of aminotransferases. The results revealed an increased dry weight of the remaining liver, regeneration rate, and DNA content at 24 and 48 hours after hepatectomy. The hepatocyte proliferation rate was significantly higher among animals treated with hyperbaric oxygen therapy at 48 hours after surgery. There was no significant difference in aminotransferase levels. Mitochondrial respiration revealed reduced oxygen consumption in state 3 after 48 hours. These results demonstrated that hyperbaric oxygen stimulates hepatic regeneration at 24 and 48 hours after 70% hepatectomy. The effect of hyperbaric oxygen on hepatic tissue occurs without tissue damage and protects mitochondria after 48 hours.

THE PROCESS OF LIVER REGENERATION that takes place after tissue loss recruits fully differentiated hepatocytes into the proliferative cycle. Although adult liver hepatocytes rarely divide under normal conditions, they may be transformed from a quiescent to a prereplication state, which is followed by DNA synthesis and mitosis, with cell division completing the sequence.[1–4] Hyperbaric oxygen therapy (HBO) is an increasingly utilized therapeutic method that employs inhalation of 100% oxygen at a pressure of more than one atmosphere in a hyperbaric chamber.[5,6]

The most important effect of HBO treatment is tissue hyperoxygenation starting from oxygen dissolved in plasma. Extracellular matrix deposition, angiogenesis, epithelialization, and bacterial phagocytosis require molecular oxygen for wound repair. The presence of O_2 is essential for lysine and proline hydroxylation, a fundamental step for collagen release by cells. Collagen maturation and binding increase linearly with the elevation of oxygen concentration in the environment.[5]

In 1966, Karasewich et al[6] reported the increased survival of dogs treated with HBO after hepatic artery ligation. Brettschneider et al[7] used HBO to preserve liver grafts in humans. Mazariegos et al[8] published a study comparing the clinical course of children undergoing liver transplantation who were or were not treated with HBO following early hepatic artery thrombosis. There was no significant difference in survival or in the rate of retransplantation. However, in the cases that required retransplantation, the procedure was performed after a longer time and under

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semi-elective conditions among the HBO-treated group. Hyperoxygenation by application of hyperbaric oxygen during the early phase of liver regeneration affects mitochondrial function and hepatocellular proliferation, possibly representing an adjuvant method for the treatment of some liver diseases. The objective of the present study was to investigate the effects of hyperbaric oxygen on liver regeneration and liver mitochondrial function 24 or 48 hours after 70% partial hepatectomy in rats.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 200 to 250 g had free access to standard laboratory chow (Purina Nutrimentos Ltda.) and water. They were kept at room temperature on 12-hour light-dark cycles according to the guidelines of the Ethics Committee of Animal Experimentation of FMRP-USP.

Partial Hepatectomy

Partial hepatectomy was performed using the technique described by Higgins and Anderson.[9] Rats were anesthetized with ether. Through a median xipho-umbilical incision, the left lateral and median liver lobes (anterior lobes), which correspond to approximately 70% of the total liver mass, were resected, while the right lateral and caudate lobes (posterior lobes) were left intact. The abdominal wall was closed layer by layer with continuous 3-0 cotton sutures.

Hyperbaric Oxygen

The groups treated with hyperbaric oxygen (HBO_2) received two applications of HBO in a monoplace chamber (Sechrist, model 2500 B) directly pressurized with oxygen in their cages. The sessions lasting 90 minutes each, with a pressure of 2 ATA were performed 1 hour and 9 hours after partial hepatectomy (separated by an 8-hour interval). They were divided into four groups.

Group I consisted of six rats who underwent partial hepatectomy and were sacrificed 24 hours later. The six rats of group II underwent partial hepatectomy plus two sessions of HBO_2 (2 ATA; 1.5 hour/session) and were sacrificed 24 hours later. Group III consisted of six rats who underwent partial hepatectomy and were sacrificed 48 hours later. Finally, group IV consisted of six rats who underwent partial hepatectomy plus 2 sessions of HBO_2 (2 ATA; 1.5 hour/session) who were sacrificed 48 hours later.

Liver Regeneration

Liver regeneration was assessed by four methods hepatocellular proliferation rate was estimated by an immunohistochemical method for proliferating cell nuclear antigen (PCNA); measurement of hepatic DNA content; dry weight of the liver remnant; and liver regeneration using the formula of Fischback.[10,11]

Mitochondrial Function

Samples of liver resected at the time of sacrifice were collected for biochemical analysis of mitochondrial function (states 3 and 4 of mitochondrial respiration); of respiratory control rate (RCR); of membrane potential; and of mitochondrial permeability transition.[12,13]

Determination of Biochemical Serum Parameters

Blood samples of approximately 4 mL were collected from each animal at sacrifice by puncture of the inferior vena cava for the determination of serum bilirubin, alkaline phosphatase, gamma-glutamyltransferase, alanine aminotransferase, aspartate aminostransferase, albumin, total proteins, and lactice dehydrogenase.[14]

Statistical Analysis

The results presented graphically were statistically analyzed by the nonparametric Mann-Whitney test with the level of significance set at 5% ($P < .05$). The statistical analysis was performed with the GraphPad Prism 4.0 software (GraphPad Software Inc, Calif).

RESULTS

Liver Regeneration

Among animals sacrificed 24 hours after partial hepatectomy, hyperbaric oxygen therapy stimulated a regenerative hepatocellular response. Their mean dry weight of the liver remnant was 1.97 mg liver/g of body weight, a significantly higher value than 1.58 mg liver/g of body weight observed in the control group. The liver regeneration rate of animals treated with HBO was double (20.24%) that of controls (9.86%). The hepatic DNA content of these animals was 90.55 μg/100 g of body weight, a significantly higher amount than 55.63 μg DNA/100 g body weight in the control group ($P < .05$). However, the hepatocellular proliferation rate, as determined by the immunohistochemical method for PCNA, was not significantly different between the two groups sacrificed 24 hours after partial hepatectomy (Fig 1).

Among the group sacrificed after 48 hours, the four analyzed variables demonstrated liver regeneration to be stimulated by HBO. Among the animals that received HBO, remnant liver dry weight was 2.62 mg liver/g body weight and liver regeneration rate was 32%, values significantly higher than the controls. (2.13 mg liver/g body weight and 26.17%, respectively). Mean liver DNA content 48 hours after partial hepatectomy was 102.03 μg/100 g of body weight in the group treated with HBO, which was significantly higher than 76.83 μg/100 g of body weight obtained for the control group. Mean proliferative hepatocellular activity, estimated by the immunohistochemical method for PCNA, was 61.87% for the HBO-treated group 48 hours after hepatectomy, a significantly higher value than the mean of 50.72% for the controls ($P < .05$; Fig 2).

Mitochondrial Function

We observed a slight increase in oxygen consumption in state 4 of mitochondrial respiration among animals treated with HBO and sacrificed 24 hours after hepatectomy compared to controls ($P < .05$). The mean value for animals treated with HBO_2 was 19.81 nanoatoms O/mg protein/min versus 12.02 nanoatoms O/mg protein/min for the controls. These values were relatively low, indicating that basal O_2 consumption by the mitochondria was low in both groups (HBO_2 and controls). There was no significant difference in state 3 oxygen consumption between the groups sacrificed at 24 hours after partial hepatectomy.

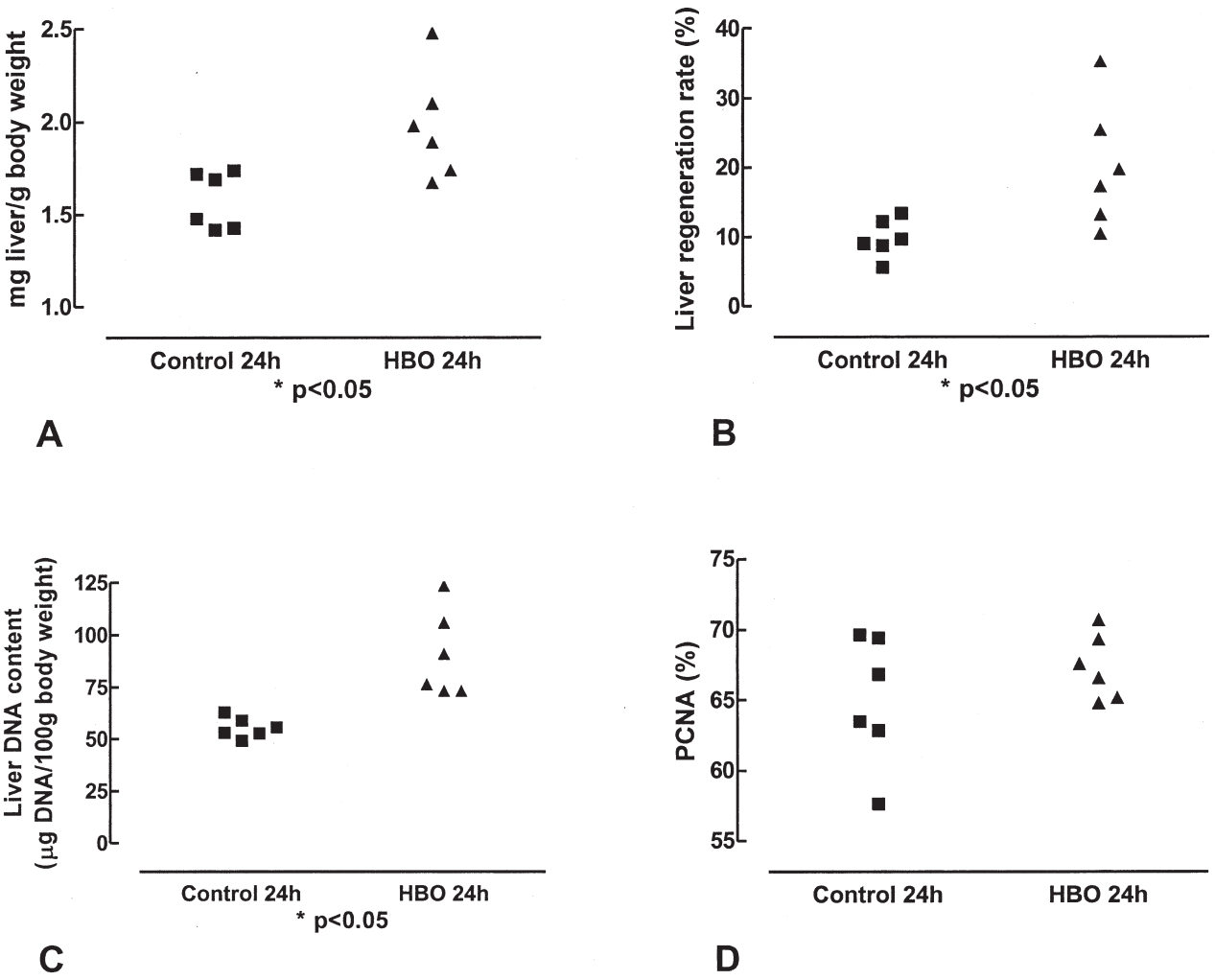

Fig 1. Graphic presentation of individual values of liver remnant dry weight **(A)**, liver regeneration rate **(B)**, liver DNA content **(C)**, and hepatocellular proliferation rate-PCNA **(D)** in HBO_2-treated animals and in controls sacrificed 24 hours after partial hepatectomy (70%). (*Controls × HBO: $P < .05$).

Among the animals sacrificed 48 hours after partial hepatectomy were the mean oxygen consumption in state 3 was 66.0 nanoatoms O/mg protein/min for those that received HBO, a significantly lower value ($P < .05$) than that observed in the controls (103.02 nanoatoms O/mg protein/min; Fig 3). There was no significant difference in state 4 oxygen consumption between controls and animals treated with HBO at 48 hours after hepatectomy.

Thus, in the present study there was a significant decrease of RCR in HBO-treated animals at 24 or 48 hours after partial hepatectomy. During the first 24 hours, the fall in RCR was due to the increased permeability of the inner mitochondrial membrane, leading to greater basal oxygen consumption (state 4). After 48 hours, the reduction in RCR was related to the lower oxygen consumption in state 3 at 24 and 48 hours after partial hepatectomy. There was no statistically significant difference in membrane electric potential or permeability transition between controls and the groups treated with hyperbaric oxygen.

Biochemical Parameters

No increase in the serum concentrations of alanine or aspartate aminotransferases was observed in the groups treated with hyperbaric oxygen. There was no significant difference in serum levels of total bilirubin, direct bilirubin, indirect bilirubin, alkaline phosphatase, total proteins, albumin, and lactic dehydrogenase between the controls and the animals treated with hyperbaric oxygen, sacrificed 24 and 48 hours after hepatectomy (Table 1).

DISCUSSION

Recent studies have established a relationship between liver regeneration and liver oxygenation. Yoshioka et al[15]

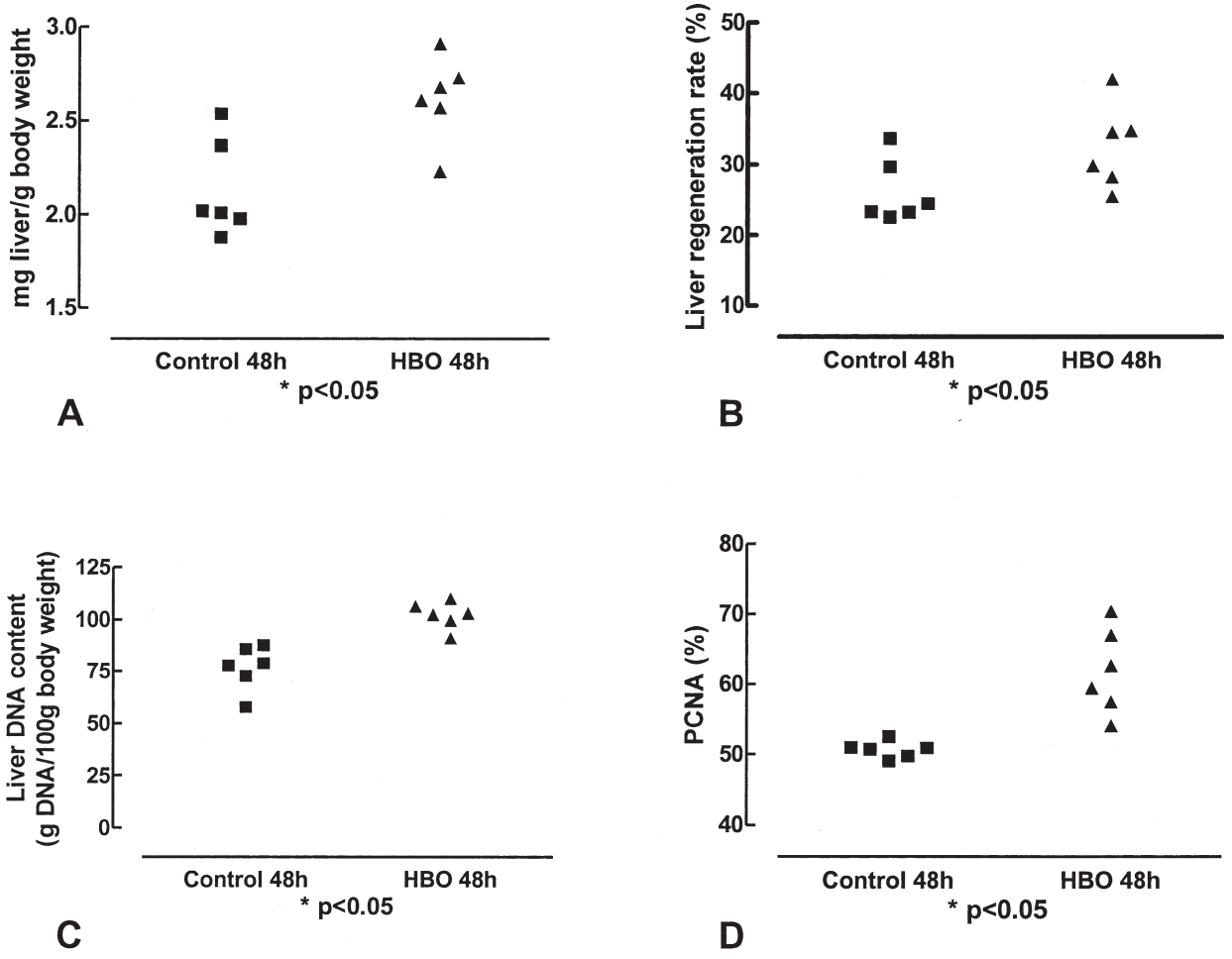

Fig 2. Graphic presentation of individual values of liver remnant dry weight **(A)**, liver regeneration rate **(B)**, liver DNA content **(C)**, and hepatocellular proliferation rate-PCNA **(D)** in HBO_2-treated animals and in controls sacrificed 48 hours after partial hepatectomy (70%). (*Controls × HBO: $P < .05$).

demonstrated that inhibition of liver regeneration by continuous octreotide infusion in hepatectomized rats led to a significant reduction in hepatic oxygen consumption and to a reduction in the usual fall in hepatic venous saturation occurring in the regenerating liver. The authors suggested that the intensity of liver regeneration was directly proportionate to the oxygen consumption by the liver.

Table 1. Serum Biochemical Parameters of Rats Submitted to Partial Hepatectomy and to 70% Partial Hepatectomy Plus Treatment With Hyperbaric Oxygen 24 and 48 Hours After Surgery

Parameter	Control (Partial Hepatectomy)	Hyperbaric Oxygen 24 Hours	Hyperbaric Oxygen 48 Hours
Total bilirubin (mg/dL)	0.43 ± 0.20	0.39 ± 0.35	0.28 ± 0.13
Direct bilirubin (mg/dL)	0.21 ± 0.12	0.23 ± 0.19	0.11 ± 0.06
Indirect bilirubin (mg/dL)	0.21 ± 0.14	0.19 ± 0.24	0.16 ± 0.08
ALT (U/L)	174.33 ± 59.70	198.33 ± 198.58	80.33 ± 16.76
AST (U/L)	299 ± 41	333.5 ± 139.86	216.33 ± 48.92
GGT (U/L)	21.16 ± 2.56	18.5 ± 2.73	20.66 ± 4.32
Alkaline phosphatase (U/L)	337.6 ± 77.5	382.8 ± 29.6	281.16 ± 20.4
Albumin (g/dL)	2.75 ± 0.13	2.91 ± 0.40	2.7 ± 0.24
Total protein (g/dL)	5.6 ± 0.38	5.21 ± 0.26	4.91 ± 0.14
LDH (U/L)	2962.5 ± 980.5	2572 ± 937	2385 ± 907.9

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; LDH = lactate dehydrogenase.

Shimizu et al[16] published an experimental study involving 85% hepatectomy accompanied by arterialization of the portal vein. Greater liver regeneration and higher ATP level in hepatic tissue were observed 7 days after partial hepatectomy. In contrast, HBO therapy also increased hepatic tissue oxygenation and, as observed in the present study, it stimulated liver regeneration, with the advantage of being a less invasive method than the arterialization.

The regenerating liver requires large amounts of energy due to metabolic overload to which it is submitted, which leads to stimulation of mitochondrial phosphorylation. Thus the liver regeneration process consumes much oxygen as a consequence of the increased energy metabolism of the mitochondria during the initial phase of liver regeneration.[16,17] Treatment with hyperbaric oxygen may stimulate liver regeneration through increased substrate oxidation and a greater ATP supply in the mitochondria.

Uwagawa et al[18] studied the effect of HBO therapy in rats previously undergoing ligation of the right branch of the portal vein, concluding that HBO-stimulated compensatory hypertrophy in the nonligated liver segments increased hepatocellular proliferation as estimated by PCNA expression, and increased serum hepatocyte growth factor levels.

Under normal conditions, mitochondria produce ATP from glucose and oxygen, but in a situation of low energy capacity, they use fatty acids and oxygen. In all situations, oxygen is required for ATP production by hepatocyte mitochondria. When liver regeneration is accelerated under normal oxygenation conditions O_2 concentration may become insufficient.[18] Tsai et al[19] reported that the changes in mitochondrial respiration immediately after partial hepatectomy were related to tissue injury produced by increased free radicals.

The velocity of state 3 reflects the mitochondrial capacity of energy production, while the velocity of state 4 reflects oxygen consumption by mitochondria after oxidative phosphorylation of ADP, corresponding to baseline respiration. RCR is an index obtained as the ratio between the velocities of states 3 and 4. This index represents the balance between the ability of mitochondria to produce energy and their energy consumption, indicating the degree of coupling between oxidation and phosphorylation. After hepatectomy there is an increase in state 3, state 4, and the phosphorylation index.

In animals that received hyperbaric oxygen and were sacrificed 48 hours after partial hepatectomy, the mean state 3 oxygen consumption was significantly lower than that in the controls. Thus the usual increase in oxygen consumption for ATP production by the mitochondria 48 hours after hepatectomy did not occur in animals treated with HBO. These results were close to the values detected in normal (nonhepatectomized) rats, demonstrating that HBO displays a protective effect on liver regeneration at 48 hours after resection.

RCR was significantly reduced among-treated HBO animals at 24 and 48 hours after partial hepatectomy. During the first 24 hours, the fall in RCR was due to increased permeability of the inner mitochondrial membrane, leading to greater baseline oxygen consumption (state 4). After 48 hours, the reduced RCR was due to the lower oxygen consumption in state 3. These results may possibly be related to the higher ATP concentrations in hyperoxygenated liver tissue as observed by other investigators.[16,20]

Table 2. Presentation of Oxygen Consumption Rate Values by Mitochondria in State 3, State 4, RCR, MP, and MPT for HBO-Treated and Control Animals Sacrificed 24 and 48 Hours After Partial Hepatectomy

Parameter	Control (Partial Hepatectomy)	Hyperbaric Oxygen 24 Hours	Hyperbaric Oxygen 48 Hours
State 3 (Activated)	84.3 ± 25.1	78.7 ± 21.2	66 ± 12.2
State 4 (Baseline)	16 ± 5.5	20 ± 4.4	16 ± 1.8
RCR	5.4 ± 0.5	3.9 ± 0.3	4.1 ± 0.4
MP	131 ± 1.7	130 ± 1.3	132 ± 1.2
MPT	0.08 ± 0.03	0.08 ± 0.02	0.07 ± 0.01

RCR, respiratory control rate; MP, membrane potential; MPT, mitochondrial permeability transition.

Oxygen consumption is reported as nanoatoms oxygen/mg mitochondrial protein per minute state 3 (Controls × HBO 48 hours; $P < .05$). RCR (Controls × HBO 24 hours and HBO 48 hours; $P < .05$).

In the present study, no increase was observed in the serum concentrations of alanine and aspartate aminotransferases in rats treated with hyperbaric oxygen, confirming that the treatment did not cause hepatocellular damage. The results of the remaining biochemical tests also did not significantly differ between the HBO and the control groups.

In summary, advances in the understanding of agents able to stimulate liver regeneration may contribute to treatment of liver diseases in the near future. Within this context, the use of hyperbaric oxygen has proven to be a viable and beneficial alternative. There was a liver-protective effect regarding mitochondrial function with increased liver regeneration after partial hepatectomy. (Table 2).

REFERENCES

1. Fausto N: Liver regeneration. J Hepatol 32:19, 2000

2. Labrecque D: Liver regeneration: a picture emerges from the puzzle. Am J Gastroenterol 89:86, 1994

3. Michalopolus GK, Defrances MC: Liver regeneration. Science 276:60, 1997

4. Koniaris LG, McKillop IH, Schwartz SI, et al: Liver regeneration. J Am Coll Surgeons 197:634, 2003

5. Chvapil M, Hurych J, Ehrlichova E: The influence of varying oxygen tensions upon proline hydroxylation and the metabolism of collagenous and noncollagenous proteins in skin slides. Z Physiol Chem 349:211, 1968

6. Karasewich EG, Harper EM, Sharp NCC, et al: Survival of dogs treated with hyperbaric oxygen following hepatic artery ligation. Surgery 59:255, 1996

7. Brettschneider L, Daloze PM, Huguet C, et al: The use of combined preservation techniques for extended storage of orthotopic liver homografts. Surg Gynecol Obstet 126:263, 1968

8. Mazariegos GV, O'Toole K, Mieles LA, et al: Hyperbaric oxygen therapy for hepatic artery thrombosis after liver transplantation in children. Liver Transpl Surg 5:429, 1999

9. Higgins GM, Anderson RM: Experimental pathology of the liver: I. Restoration of the liver of the white rat following partial surgical removal. Arch Pathol 12:186, 1931

10. Assy N, Gong Y, Zhang M, et al: Use of proliferating cell nuclear antigen as a marker of liver regeneration after partial hepatectomy in rats. J Lab Clin Med 131:251, 1998

11. Ramalho FS, Ramalho LN, Castro-E-Silva O Jr, et al: Effect of angiotensin-converting enzyme inhibitors on liver regeneration in rats. Hepatogastroenterology 49:1347, 2002

12. Souza MEJ, Polizello ACM, Uyemura SA, et al: Effect of fluoxetine on rat liver mitochondria. Biochem Pharmacol 48:535, 1994

13. Jordani MC, Santos AC, Prado IMR, et al: Flufenamic acid as an inducer of mitochondrial permeability transition. Mol Cell Biochem 210:153, 2000

14. Henry JR, Chiamori N, Golub OJ, et al: Revised spectrophotometric methods for the determination of glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, and lactic dehydrogenase. Am J Clin Pathol 34:381, 1960

15. Yoshioka S, Miyazaki M, Shimizu H, et al: Hepatic venous hemoglobin oxygen saturation predicts regenerative status of remnant liver after partial hepatectomy in rats. Hepatology 27:1349, 1998

16. Shimizu Y, Miyazaki M, Shimizu H, et al: Beneficial effects of arterializations of the portal vein on extended hepatectomy. Br J Surg 87:784, 2000

17. Brown GC: Control of respiration and ATP synthesis in mammalian mitochondria and cells. Biochem J 284:1, 1992

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

19. Tsai JL, King KL, Chang CC, et al: Changes of mitochondrial respiratory functions and superoxide dismutase activity during liver regeneration. Biochem Int 28:205, 1992

20. Chen M-F, Chen H-M, Ueng SWN, et al: Hyperbaric oxygen pretreatment attenuates hepatic reperfusion injury. Liver 18:110, 1998