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The effects of hyperbaric oxygen application against cholestatic oxidative stress and hepatic damage after bile duct ligation in rats

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ABSTRACT

Background: The aim of this study was to evaluate the preventive and therapeutic potential of hyperbaric oxygen therapy (HBO) on the liver tissue against bile duct ligation (BDL)-induced oxidative damage and fibrosis in rats.

Materials and methods: We divided 32 adult male Sprague Dawley rats into four groups: sham, sham plus HBO, BDL, and BDL plus HBO; each group contained eight animals. We placed the sham plus HBO and BDL plus HBO groups in an experimental hyperbaric chamber in which we administered pure oxygen at 2.5 atmospheres absolute 100% oxygen for 90 min on 14 consecutive days.

Results: The application of BDL clearly increased the tissue malondialdehyde level, myeloperoxidase activity, and hydroxyproline content and decreased the antioxidant enzymes (superoxide dismutase and catalase activities) and glutathione level. Hyperbaric oxygen therapy treatment significantly decreased the elevated tissue malondialdehyde level, myeloperoxidase activity, and hydroxyproline content and increased the reduced superoxide dismutase and catalase activities and glutathione level in the tissues. The changes demonstrating the bile duct proliferation and fibrosis in expanded portal tracts include the extension of proliferated bile ducts into lobules, mononuclear cells, and neutrophil infiltration into the widened portal areas were observed in BDL group. Treatment of BDL with HBO attenuated alterations in liver histology. Alpha smooth muscle actin, cytokeratin-positive ductular proliferation, and the activity of terminal deoxynucleotidyl transferase 2′-deoxyuridine, 5′-triphosphate nick end labeling in the BDL decreased with HBO treatment.

Conclusions: The data indicate that HBO attenuates BDL-induced oxidative injury, hepatocytes damage, bile duct proliferation, and fibrosis. The hepatoprotective effect of HBO is associated with antioxidative potential.

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1. Introduction

Extrahepatic biliary obstruction is associated with high rates of postoperative morbidity and mortality. Regardless of its origin, it is associated with morphological and functional damage of the liver [1,2]. This condition causes known biochemical, pathophysiological, and morphologic abnormalities produced by interrupted enterohepatic circulation, obstructed hepatic biliary tree, increased biliary pressure, retention of biliary constituents, and impairment of hepatocellular transport. In some circumstances, such as biliary atresia, despite early diagnosis and prompt surgical intervention to improve biliary drainage, most patients will either require liver transplantation or die as a result of progressive liver fibrosis [3–5].

Cholestasis produced by bile duct ligation (BDL) in rats induces a number of physiological changes, including obstructive jaundice, secondary liver cirrhosis, and fibrosis [6]. How cholestasis induces liver injury and fibrosis remains unclear. Hepatic fibrosis, the main complication of chronic liver disease, is usually initiated by hepatocyte damage, leading to recruitment of inflammatory cells and platelets, activation of Kupffer cells, and subsequent release of cytokines and growth factors [7]. These factors probably link the inflammatory processes with oxygen free radicals, which are known to cause tissue fibrosis [8,9]. It is known that the increased concentration of bile acids induces lipid peroxides, probably related to the stimulation of phagocytic activity in the polymorphonuclear leukocytes and inflammatory cells, which are present after biliary tract obstruction and enhance tissue injury [10,11]. Thus, free radical ablation for the treatment of cholestatic liver injury might be useful in preventing fibrosis and oxidative damage after biliary obstruction.

Hyperbaric oxygen therapy (HBO) is a specific type of oxygen administration that aims to improve numerous kinds of hypoxic disorders by increasing the amount of dissolved oxygen within the blood [12]. Hyperbaric oxygen therapy has been able to improve the evolution of animals and human beings with several diseases, in the physiopathologies of which are triad, hypoxia, ischemia, and reperfusion. Its application and indication in the treatment of several diseases are scientifically assured. It can even be used with specific diseases, such as peripheral vascular diseases [13–15]. Nevertheless, there is a vast field in the medical arena in which its usefulness and unfavorable effects are still unknown; this stimulates clinical and experimental studies, controlled *in vitro* or *in vivo*, to evaluate the desirable effects and possible complications [13,16].

Many studies reported that anti-inflammatory and antioxidant drugs may show beneficial effects on persistent inflammation and oxidative damage in cholestasis [17–20]. However, most of these drugs are in experimental processes and are not ready for clinical use. We designed this study to test the hypothesis that HBO could be useful in the treatment of BDL-induced oxidative damage and fibrosis in rats.

2. Materials and methods

2.1. Animals

In this study, we used 32 healthy male Sprague Dawley rats, weighing 250–300 g and averaging 12 wk of age. Food and tap water were available *ad libitum*. In the windowless animal quarter, automatic temperature (21°C ± 1°C) and lighting controls (12 h/12 h light-dark cycle) were maintained. Humidity ranged from 55% to 60%. All animals received human care according to the criteria outlined in the *Guide for the Care and Use of Laboratory Animals*, prepared by the National Academy of Sciences and published by the National Institutes of Health.

2.2. Experimental groups and protocols

We used 32 Sprague Dawley adult male rats in this study and divided them into four groups: sham, sham plus HBO, BDL, and BDL plus HBO; each group contained eight rats.

We deprived all rats of food for 1 d before surgery. We intraperitoneally anesthetized the rats with ketamine (50 mg/kg) and xylazine (5 mg/kg). The common bile ducts (CBDs) of rats in the sham and sham plus HBO groups were uncovered after being inserted into the abdomen by means of midline abdominal incision. Then, we closed the abdominal wall with a 2–0 silk suture. We injected BDL and BDL plus HBO into the abdominal cavity in a similar way and exposed the CBDs. The CBDs were located and obstructive jaundice was induced by double ligation with 4–0 silk and transection of the CBDs in the supraduodenal part between the lowermost tributary of the bile duct and the uppermost tributary of the pancreatic duct. In the sham plus HBO and BDL plus HBO groups, we placed rats into a hyperbaric chamber designed for small experimental animals (model ht an_01; Hipertech Co. Ltd.; Istanbul, Turkey) and then pressurized it to 2.5 atmospheres absolute with 100% oxygen for 90 min for 14 consecutive days. We performed compression and decompression at a rate of 0.15 atmospheres absolute/min.

After 2 wk of treatment, we decapitated the rats. After we opened opening the abdominal cavity, we immediately immersion fixed the liver tissue samples in Bouin's solution for histologic evaluation or stored them at −80°C for subsequent spectrophotometric determination of activity of superoxide dismutase (SOD) and catalase (CAT) and levels of malondialdehyde (MDA) and glutathione (GSH). We found indirect evidence of neutrophil infiltration by measuring tissue-associated myeloperoxidase (MPO) activity, and assessed collagen accumulation by measuring hydroxyproline (HP) content in liver samples.

2.3. Biochemical measures

We measured liver MDA levels using the thiobarbituric acid reaction according to the method of Buege and Aust [21]. We used this method to obtain a spectrophotometric measurement of the color produced during the reaction to thiobarbituric acid with MDA at 535 nm.