Effects of hyperbaric oxygen therapy on the liver after injury caused by the hepatic ischemia-reperfusion process

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ABSTRACT

PURPOSE: To evaluate the effects of hyperbaric oxygen on rats submitted to hepatic ischemia and reperfusion.

METHODS: Twenty-three Wistar rats were divided at random into 3 groups: SHAM, rats submitted to surgical and anesthetic stress without induction of hepatic ischemia/reperfusion; I/R, rats submitted to total ischemia of the hepatic pedicle for 25 min followed by 5 min of reperfusion; HBOI/R, rats submitted to 60 min of hyperbaric oxygen therapy at a pressure of 2 absolute atmospheres immediately after the experimental protocol of ischemia/reperfusion. Hepatic function was evaluated by quantitation of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and by mitochondrial function through the determination of states 3 and 4 of mitochondrial respiration, respiratory control ratio (RCR) and mitochondrial swelling. Data were analyzed by the Mann-Whitney test, with the level of significance set at p <0.05.

RESULTS: There was a significant difference in state 3 values for the SHAM group vs I/R and I/R vs IRHBO, in state 4 values for the SHAM group vs I/R; and in mitochondrial swelling for the SHAM groups vs IRHBO, SHAM vs I/R, and IR vs IRHBO.

CONCLUSION: The use of hyperbaric oxygen after I/R improved in a relative manner both the production of energy and the effects on the mitochondrial wall.

Key words: Mitochondria. Ischemia. Reperfusion. Hyperbaric oxygen therapy. Liver.
Introduction

Hyperbaric oxygen therapy (HBO) is a treatment based on high concentrations of partial oxygen pressure inside a compartment known as hyperbaric chamber. The oxygen used in the chamber is pure and is present at a pressure of two atmospheres. Among the multiple indications of HBO are osteomyelitis, acute vasculites caused by medications and biological toxins, and gaseous gangrene, in addition to cases of acute anemia in which blood transfusion is not possible.

The ischemia-reperfusion (I/R) process is essential for certain surgical procedures such as partial liver resections and organ transplantation. Liver transplantation is a condition during which the injury generated by this process is observed, with its intensity and types of prevention and treatment still being controversial subjects.

It is believed that submission of tissues and organs to a high oxygen concentration after I/R may improve or at least alleviate the effects of ischemia and, mainly, reperfusion. Tissue hypoxia is considered to generate certain elements that alter the target organ anatomically and functionally and these elements are considered to be exacerbated by tissue reperfusion, aggravating the hepatic injury. Ischemia is related to the triggering of a series of biochemical reactions that ultimately injure the hepatic tissue, while reperfusion is related to the generation of free radicals, among other factors. The I/R process involves changes ranging from microvascular alterations to the activation of neutrophils, platelets, Kupffer cells and sinusoidal endothelial cells. Thus, the objective of the present study was to investigate the effect of the use of HBO after an I/R process.

Methods

Twenty-three male Wistar rats weighing 200 to 300 g were used. The animals were divided into three groups: SHAM – rats submitted to surgical and anesthetic stress without exposure to HBO or clamping of the hepatic pedicle; I/R – rats submitted to 25 min of ischemia followed by 5 min of reperfusion, with no exposure to HBO; I/R-HBO – rats submitted to 25 min of ischemia and 5 min of reperfusion, followed by 60 min of HBO at 2 absolute atmospheres (ATA).

Duly cleaned but not sterilized surgical material was used for the operation in a standardized manner. The animals were anesthetized with a solution of xylazine hydrochloride (20 mg/ml) and ketamine hydrochloride (50 mg/ml) at a 1:2 proportion, administered at the dose of 100 mg/kg body weight. A median laparotomy was performed, extending from the inferior third of the xiphoid appendix to the pubis in the superoinferior direction, followed by exploration of the abdominal cavity, delicate dissection of the round ligament of the liver and identification of the hepatic pedicle. The hepatic pedicle was fully clamped for 25 min with a home-made clamp. The animal was then submitted to 5 min of reperfusion and sacrificed by total exsanguination by puncture of the inferior vena cava after exposure of the vein by manipulation of the abdominal viscera.

The HBO procedure was based on the simultaneous exposure of three rats to oxygen at an atmospheric pressure of 2 ATA in a hyperbaric chamber (Sechrist, modelo 2500 B) directly pressurized with oxygen. Each session lasted 60 min, divided into 15 min of compression followed by 30 min of exposure to high-pressure oxygen and 15 min of chamber decompression, corresponding to 30 uninterrupted min of HBO at 2 ATA.

Hepatic function was determined by measuring serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) by analysis of the following parameters of mitochondrial function in hepatic tissue: states 3 and 4 of mitochondrial respiration, respiratory control ratio (RCR), and mitochondrial swelling.

The results of the biochemical tests were analyzed statistically by the nonparametric Mann-Whitney test, with the level of significance set at 5% (p<0.05). The results were analyzed statistically using the Prisma GraphPad 4.0 software (GraphPad Software Inc, CA).

Results

Analysis of the values presented in Figure 1 revealed that there was a difference in the four parameters investigated according to the process to which the animals were submitted.
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As shown in Figure 1, it can be seen that the rate of oxygen consumption and the consequent production of energy (State 3) in the form of adenosine triphosphate (ATP) differed significantly between groups (SHAM vs IR and IR vs IR+HBO), being more exacerbated in the SHAM and IR groups compared to the IR and IRHBO groups. The rate of oxygen consumption in the basal state, represented by the graph for state 4, differed significantly between the SHAM and IR groups, since the SHAM and IR+HBO groups and the IR and IR+HBO groups did not show a difference. Regarding mitochondrial swelling, the associations between SHAM and IR, SHAM and IR+HBO, and IR and IR+HBO groups did not show a difference. The RCR did not differ between groups.

**Discussion**

The present study intended somehow to clarify a topic of extreme importance such as hepatic injury due to I/R. This complex that affects the liver in various surgical procedures such as liver transplantation is being investigated in order to find some way of attenuating its adverse effects. In an attempt to contribute to a greater number of possibilities for the attenuation of the undesirable effects of the procedure, we performed the surgical procedure of hepatic I/R followed by submission of the experimental animals to high oxygen pressures in a hyperbaric chamber. Previous clinical studies by our group have shown a beneficial effect of HBO for the treatment of patients with post-liver transplant complications. The experiment involved the analysis of parameters related to mitochondrial respiration such as State 3, State 4 and mitochondrial swelling related to calcium homeostasis and RCR.

In the present study, the production of energy by the mitochondria was analyzed starting from State 3. Regarding this analysis, we may state that the animals submitted to HBO after hepatic I/R showed an improvement when compared to the rats submitted only to I/R. This demonstrates that the capacity of respiration and, consequently, of energy production was improved in animals submitted to higher oxygen pressure after clamping and unclamping of the hepatic pedicle. In contrast, comparison of the IR group to the SHAM group revealed a reduced capacity of energy production, which was preserved in the HBO+IR group. On this basis, we may conclude that HBO theoretically attenuated the
effects of the period of ischemia due to the promotion of aerobic metabolism by preserving the ATP reserves\textsuperscript{1}.

Regarding State 4 of mitochondrial respiration, there was an expressive difference between the SHAM and IR groups (p<0.05), with a reduced oxygen consumption in the IR group due to interruption of blood flow. Although expected, no difference was observed with HBO. This fact was not observed in other studies, in which the SHAM and IR groups already presented some difference\textsuperscript{10,12,16}. In those studies there was an increase in the rate of oxygen consumption by the mitochondria compared to the SHAM group in order to maintain the electrochemical proton gradient on the mitochondrial membrane so that the membrane would be able to phosphorylate ADP\textsuperscript{16,17}.

RCR also did not differ significantly between the groups studied. Treatment under hyperoxia did not demonstrate an improvement compared to the group submitted to IR. Regarding this parameter, the SHAM group (p>0.05) was practically similar to the IR group.

Regarding the swelling of the mitochondrial matrix, considerable differences were detected between the SHAM, IR and IR+HBO groups. This parameter is induced by calcium and phosphate ions which act on this process related to cell permeability. In this respect, it was observed that there was less swelling in the IR and IR+HBO groups than in the SHAM group. However, the IR+HBO group was found to be closer to the SHAM group, leading us to conclude that the wall injury was attenuated by HBO, since the group was comparable to the IR group, whose reduced absorbance induced by calcium and phosphate ions which act on this process related to cell permeability. In this respect, it was observed that there was less swelling in the IR and IR+HBO groups than in the SHAM group.

### Conclusion

Experiments involving ischemia/reperfusion followed by hyperbaric oxygen revealed a relative improvement of the functional parameters of hepatic mitochondria both regarding energy production and damage to the mitochondrial wall.

### References

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