Effect of hyperbaric hepatic hyperoxia on the liver of rats submitted to intermittent ischemia/reperfusion injury[1]

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ABSTRACT

PURPOSE: To determine the effect of hyperbaric hyperoxia as hepatic preconditioning on hepatocellular integrity in rats submitted to intermittent hepatic ischemia/reperfusion injury.

METHODS: Twenty male Wistar rats were divided into 4 groups (SHAM, I/R, HBO-I/R and CONTROL). The surgical technique consisted of total clamping of the hepatic pedicle for 15 min, followed by reperfusion for 5 min, performed twice. The application of hyperbaric oxygen (HBO) was carried out in a collective chamber (simultaneous exposure of 4 rats) pressurized directly with oxygen at 2 ATA for 60 min. Tissue malondialdehyde (MDA) levels were determined and blood samples were collected for the determination of serum AST and ALT levels. Data were analyzed statistically by the Mann-Whitney test, with the level of significance set at $p < 0.05$.

RESULTS: A statistically significant difference in MDA ($p< 0.05$) was observed between control and HBO-I/R, but not between control and I/R. Regarding AST, there was a difference between control and I/R and HBO-I/R. Analysis of ALT revealed a significant difference between control and I/R ($p<0.05$) and between I/R and HBO-I/R, with no difference between control and HBO-IR.

CONCLUSION: Hyperoxic preconditioning proved to be favorable regarding alanine transaminase, but not aspartate aminotranserase or malondialdehyde levels.

Key words: Hyperbaric Oxygenation. Liver. Ischemia. Reperfusion. Lipid Peroxidation. Rats.

Introduction

In hepatic ischemia/reperfusion (I/R), hepatic damage occurs by temporary deprivation of blood flow to the liver, as observed in hepatectomy, liver transplantation and hemorrhagic shock, with consequent effects ranging from a slight increase in serum levels of the hepatocellular enzymes aspartate aminotransferase (AST) and alanine transaminase (ALT) to acute liver failure. During the period of ischemia followed by reperfusion there is a complex interaction among microvascular changes, the release of inflammatory mediators, reactive oxygen species (ROS), and neutrophil, platelet, Kupffer cell and sinusoidal endothelial cell activation[1-7]. As blood flow to the injured organ is reestablished, the injury suffered during the period of ischemia is aggravated. Oxidative phosphorylation is a process severely affected by reperfusion; since the respiratory chain is located on the inner mitochondrial membrane and is an important target of the injury caused by ROS originated during the period of reperfusion, it is natural to assume that a change in mitochondrial membrane permeability will result in impairment of oxidative phosphorylation. On this basis, a mitochondrial permeability transition occurs, characterized by a nonselective increase in the permeability of the inner mitochondrial membrane followed by osmotic swelling[5,8-10].

Aminotransferases are intracellular enzymes that catalyze the reversible transfer of an alpha-amino group from an amino acid to an alpha-ketoacid, with the formation of new alpha-amines and alpha-ketoacids. ALT and AST are enzymes synthesized in the liver, kidney, cardiac and skeletal muscle, brain and pancreas. AST is mainly cytoplasmic and, at a lower concentration, mitochondrial, while ALT is exclusively cytoplasmic, with its values being indicators of hepatocellular integrity. Thus, serum ALT level is associated with acute hepatocellular involvement, its biochemical determination is of high diagnostic and prognostic value and changes in its serum levels are related to organic dysfunction[4,11]. The effect of hyperbaric oxygen therapy (HBO) on I/R is a matter of controversy due to the lack of basic research aiming at the determination of its mechanism of action. In a previous study by our group we showed that hyperbaric pre-conditioning did not improve significantly hepatic mitochondrial function after I/R[9,10,12]. Thus, in the present study we investigated experimentally whether HBO used as preconditioning might prevent hypoxic hepatic suffering by attenuating the injury caused by intermittent ischemia.

Methods

Research approved by the Ethics Committee for Animal Experimentation of FMRP-USP.

Twenty male Wistar rats weighing 250 to 300 g were divided into four groups of 5 animals each as follows: SHAM – rats submitted to surgical and anesthetic stress without exposure to HBO and without hepatic pedicle clamping during the same I/R period as a quality control of the mitochondrial respiration assay; I/R – rats twice submitted to 15 min of ischemia followed by 5 min of reperfusion without exposure to HBO (40 min); HBO-I/R – rats submitted to 60 min of HBO at 2 absolute atmospheres (ATA) immediately followed by two periods of 15 min of ischemia and 5 min of reperfusion (40 min); control – rats submitted to immediate blood and hepatic tissue collection without I/R and HBO.

The rats were housed in the animal facilities of FMRP-USP at room temperature, with a sleep-wakefulness cycle of 12h and with free access to laboratory ration (Purina Nutrimentos Ltda.) and water.

Surgical technique

The animals were anesthetized intramuscularly with 20 mg/ml solution of xylazine hydrochloride and a 50 mg/ml solution of ketamine hydrochloride at a 1:2 proportion of 100 mg/kg/ weight. The surgical procedure started with a median laparotomy extending from the lower third of the xiphoid appendix to the pubis, followed by exploration of the abdominal cavity, delicate dissection of the round ligament of the liver and identification and exposure of the hepatic pedicle. The pedicle was twice fully clamped with a home-made clamp for 15 min, followed by 5 min of reperfusion (declamping), for a total cycle of 40 min. The animal was sacrificed by total exsanguination by puncture of the inferior vena cava after its exposure by manipulation of the abdominal viscera[12].

HBO was performed in a collective chamber (simultaneous exposure of 5 rats) (Sechrist, model 2500 B) directly pressurized with oxygen. Each session lasted 60 min, 15 of which were devoted to compression and 15 to decompression inside the chamber, with the animals thus being submitted to 30 uninterrupted min of HBO at 2 ATA[9,10]. Presurgical exposure to HBO and all surgical procedures were always performed during the same period of the day.

Determination of tissue malondialdehyde (MDA)

MDA was determined by a colorimetric method based on reaction with thiobarbituric acid at 532 nm using a Versamax microplate reader (Molecular Devices) and 1,1,3,3-tetramethoxypropane (0 to 100 μm) as the standard. The results obtained are reported as μM/mg protein[13].

Determination of serum AST and ALT

AST and ALT were determined by a kinetic method using a CELM spectrophotometer at 340 nm. The results are reported as U/L[4,14].

Statistical analysis

Data were analyzed statistically by the nonparametric Mann-Whitney test, with the level of significance set at 5% or less ($p<0.05$), using the GraphPad Prism 5 software (GraphPad Software Inc, CA).

Results

Regarding MDA levels, the control group differed significantly ($p<0.05$) from the HBO-IR but not from the I/R group ($p>0.05$). There was no difference between the I/R and HBO-IR groups ($p>0.05$) or between the control and Sham groups (Figure 1).

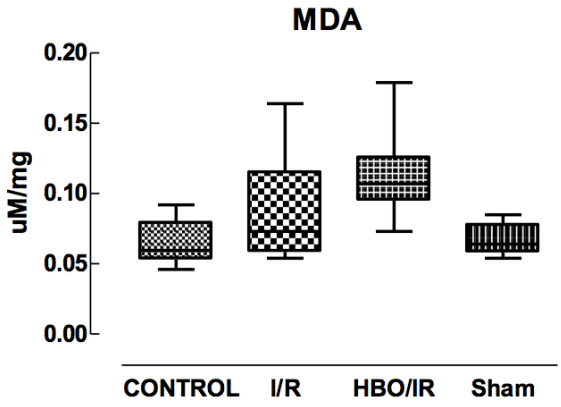

FIGURE 1 - Tissue levels of malondialdehyde (MDA): control (rats submitted to immediate blood and hepatic tissue collection without I/R or HBO). I/R (rats twice submitted to 15 min of ischemia followed by 5 min of reperfusion (40 min), without exposure to HBO). HBO-I/R (rats submitted to 60 min of HBO at 2 absolute atmospheres and then immediately submitted twice to 15 min of ischemia followed by 5 min of reperfusion (40 min). Sham (rats submitted to surgical stress without exposure to HBO and without clamping of the hepatic pedicle for 40 min). Control vs I/R ($p> 0.05$), control vs HBO-IR ($p< 0.05$), control vs Sham ($p>0.05$), I/R vs HBO-IR ($p> 0.05$), Sham vs I/R ($p>0.05$), and Sham vs HBO-IR($p< 0.05$).

Regarding AST, the control group differed significantly from the I/R and HBO-IR groups ($p< 0.05$). There was no difference between the control and Sham groups ($p>0.05$) or between the I/R and HBO-I/R groups ($p> 0.05$) and between the Sham and I/R and HBO-I/R groups ($p> 0.05$) (Figure 2).

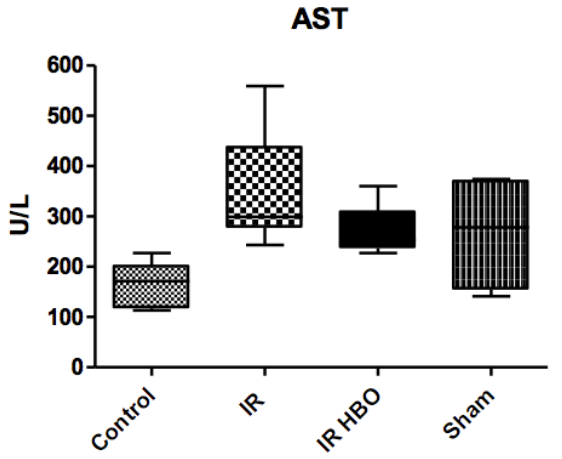

FIGURE 2 – Tissue levels of aspartate aminotransferase (AST). Control (rats submitted to immediate blood and hepatic tissue collection without I/R or HBO). I/R (rats twice submitted to 15 min of ischemia followed by 5 min of reperfusion (40 min), without exposure to HBO). HBO - I/R (rats submitted to 60 min of HBO at 2 absolute atmospheres and then immediately submitted twice to 15 min of ischemia followed by 5 min of reperfusion (40 min). Sham (rats submitted to surgical stress without exposure to HBO and without clamping of the hepatic pedicle for 40 min). Control vs I/R and HBO-IR ($p< 0.05$), Control vs Sham ($p>0.05$), I/R vs HBO-IR ($p> 0.05$), Sham vs I/R and HBO-IR($p> 0.05$).

Analysis of ALT revealed a significant difference between the control and I/R groups ($p<0.05$) but not between the control and HBO-I/R groups ($p> 0.05$). There was no difference between the control and Sham groups ($p>0.05$), but there was a difference between the I/R and HBO-I/R groups ($p< 0.05$). There was no significant difference between the Sham and I/R or HBO-I/R groups ($p> 0.05$) (Figure 3).

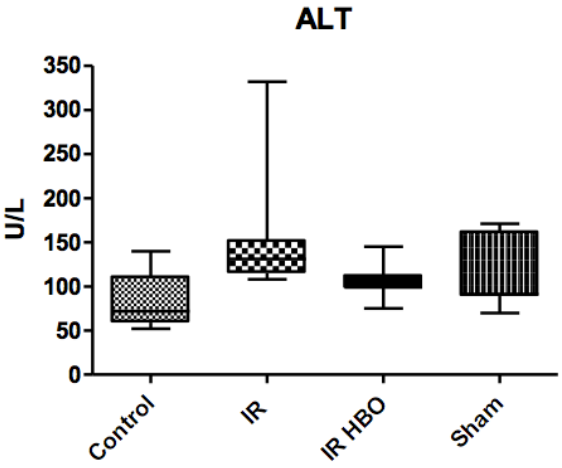

FIGURE 3 – Tissue levels of alanine aminotransferase (ALT). Control (rats submitted to immediate blood and hepatic tissue collection without I/R or HBO). I/R (rats twice submitted to 15 min of ischemia followed by 5 min of reperfusion (40 min), without exposure to HBO). HBO - I/R (rats submitted to 60 min of HBO at 2 absolute atmospheres and then immediately submitted twice to 15 min of ischemia followed by 5 min of reperfusion (40 min). Sham (rats submitted to surgical stress without exposure to HBO and without clamping of the hepatic pedicle for 40 min). control vs I/R ($p<0.05$), control vs HBO-IR ($p> 0.05$), Control vs Sham ($p>0.05$), I/R vs HBO-IR ($p< 0.05$), Sham vs I/R and HBO-IR($p> 0.05$).

Discussion

Hepatic I/R injury has been extensively studied in the literature using serum and tissue indicators[5,12,15,16]. Regarding mitochondrial function, some studies have shown beneficial and protective effects of HBO therapy in the rat liver, although others have not demonstrated this efficacy[9,10,17]. Some authors have stated that exposure to a higher pressure increases oxygen content in plasma and in body tissues, possibly normalizing oxygen levels in the ischemic tissues. This has led to questioning whether the oxygen made available by the chamber would be utilized to aggravate the injury by providing a geater substrate for the formation of free radicals or whether this normalization would contribute to mitochondrial and tissue homeostasis. It was first thought that HBO migh exacerbate the injury, increasing the oxygen supply to the organism, with an increased production of free radicals. Although experimental studies on rats submitted to I/R have demonstrated the beneficial effects of HBO during reperfusion, there is no consensus in the literature[9,10,17,18]. In the present study, with respect to MDA, we observed a tendency to an increase of ROS in the I/R group, which was aggravated in the HBO-I/R group, demonstrating a lack of protection, as also reported in other studies[9,10,19].

The absolute serum levels of ALT and AST and/or the ALT/AST ratio may serve as indicators of hepatocellular integrity, with an association between the increased serum levels of these enzymes and acute liver involvement[4]. No significant improvement occurred in AST levels, with no significant difference between the groups submitted or not to HBO, and with the levels of both groups being higher than those of the control group. This leads us to question the hepatoprotective effect of hyperoxic preconditioning, which is supported by some authors and contested by others[9,10,19,20].

ALT levels differed significantly between the control and I/R groups, indicating an increase in the enzyme in non-preconditioned tissue, whereas HBO-preconditioned tissue was protected by the procedure. In agreement with other literature reports, the present study demonstrates that hyperoxic preconditioning has a hepatoprotective effect, reducing the total levels of this enzyme in the HBO-I/R group to levels similar to those of the control group. This protective effect on ALT levels agrees with the results of osmotic mitochondrial swelling, with a reduction of mitochondrial edema observed in the groups submitted to preconditioning in a previous study[9,10].

These results are scientifically conflicting since some authors have suggested that the combination of HBO and I/R is positive while others consider it to be negative. These data suggest that, during the initial phase of I/R injury, which occurs immediately after reperfusion, hyperoxia attenuates the damage of the I/R process regarding ALT. The different findings for AST and ALT may be explained by the specificity of ALT in hepatic tissue. The increase in serum AST may occur due to surgical exposure and its harmful repercussions on skeletal tissue and on cardiac/hemodynamic factors, for example, during the experimental procedure[21-23].

The two five minute periods of reperfusion studied were probably insufficient for the occurrence of an effective hyperoxic preconditioning and therefore they did not prevent hepatocellular damage, except for ALT, which was present in acceptable levels close to those of the control group. Although studies involving continuous, non-intermittent, I/R injury have shown that hyperbaric preconditioning improves hepatic mitochondrial function and reduces the serum markers of liver injury in the I/R process, it is known that repeated I/R episodes can be more harmful than continuous ischemia[24].

Thus, we suggest that the procedure of intermittent I/R, by being more damaging, did not permit HBO to be equally effective, except for the ALT enzyme, which showed significant improvement in the group submitted to HBO. We believe that further studies regarding the generation of ROS and the production of nitric oxide might complement the elucidation of the possible causes of this difference.

Conclusion

Hyperoxic preconditioning proved to be favorable regarding alanine transaminase, but not aspartate aminotranserase or malondialdehyde levels.

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