Hyperoxic preconditioning in partial liver ischemia[1]

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ABSTRACT

PURPOSE: To evaluate the effect of the hyperbaric oxygen (HBO) treatment as a pre-conditioning for I/R effects in the liver ischemia.

METHODS: Fifty-seven male Wistar rats (260-300g) were submitted to the following procedures: SHAM; I/R, rats submitted to I/R, consisting of partial ischemia of 70% of the liver for 90 minutes followed by 15 minutes of reperfusion; HBO I/R 1 ATA, 30 minutes of HBO treatment at the pressure of 1 absolute atmosphere (ATA) during the ischemia time. HBO I/R 2 ATA, 30 minutes of HBO (2 ATA) during the ischemia time. Pre HBO I/R 30', rats submitted to 30 minutes of HBO (2 ATA) immediately before the I/R time. Pre HBO I/R 90', rats submitted to 90 minutes of HBO (2 ATA) immediately before the I/R time.

RESULTS: There was a significant worsening of all the parameters of mitochondrial energy production (state 3, 4, RCR and Swelling) in the I/R group, when compared to the Sham group (I/R <Sham, $p<0.05$). There was also a significant worsening in state 4, RCR and mitochondrial edema in the Pre HBO I/R 90' group compared to the I/R group. Hepatic enzyme concentrations were significantly higher in the I/R group.

CONCLUSION: The use of hyperbaric oxygen before and during I/R did not improve the production of hepatocellular energy reduced by I/R, nor did it prevent the installation of mitochondrial edema induced by Iischemia/reperfusion.

Key words: Hyperbaric Oxygenation. Mitochondria. Liver. Ischemia. Reperfusion. Rats.

Introduction

In ischemia-reperfusion (I/R), hepatic injury occurs due to temporary deprivation of blood flow to the liver, as observed in hepatectomies and in liver transplants. The ischemic preconditioning has been proved to improve the tissue response to ischemia during procedures such as mentioned before, because of the induced adaptation of the liver to the ischemic environment[1,2]. These facts helped develop some very important techniques in liver surgery, such as the Pringle Technique[3,4]. The reperfusion process is essential for the restoration of the tissue function, and can reverse the damage depending on the duration of the ischemia. Without reperfusion, the absence of oxygen causes reduction of the respiratory chain, instead of oxidation. Though the restoration of blood flow to an organ may prevent irreversible cell damage, it is known that it's effects are even more lethal to the organ than the ischemia itself[5]. The damage caused by ischemia in the liver is due to microvascular alterations such as increased adhesion and activation of leukocytes, Kupffer cells and platelets, depletion of adenosine triphosphate, complement activation, liberation of calcium and reactive oxygen species formation[6]. The oxidative phosphorylation process is also severely affected by reperfusion, because of its location in the inner mitochondrial membrane, which is highly damaged by the oxygen reactive species. Therefore, damage caused to the membrane will result in a less efficient oxidative phosphorylation, such as occurs in the reperfusion process, because of an alteration in the membrane permeability and consequent mitochondrial swelling[7]. The effects of the I/R vary from alterations of hepatic enzymes to acute liver failure, depending on its duration[8,9].

Hyperbaric oxygen (HBO) is a specific type of treatment of several hypoxia disorders consisting of the use of oxygen under hyperbaric conditions, increasing the quantity of oxygen dissolved in blood and in body tissues (Henry's Law) and promoting high tissue oxygen tension[10]. It is known that this treatment operates with two physical factors of the hyperbaric chamber: effects of mechanical stress and increased tissue oxygenation[11]. The use of HBO in the I/R damage is controverse, because it was believed that its use would increase tissue damage by raising the level of oxygen and consequently the level of reactive species and free radicals in the blood. However, there are studies that show positive changes caused by its use, suggesting a protective effect in different tissues[10-12]. There are also studies that suggest an aggravation of tissue damage[8] or no significant effect[7].

The objective of this study is to evaluate the effect of the HBO treatment as a pre-conditioning for I/R and HBO effects at the same time of ischemia.

Methods

The project was approved by the Ethics Committee of University of São Paulo.

Fifty-seven male Wistar rats weighing between 260 and 300g, from the general animal house of the Experimental Surgery of the Ribeirão Preto Medical School of the University of São Paulo were studied. The animals were fed with standard laboratory diet for the species and water at will.

The animals were divided in six groups and submitted to the following procedures: SHAM, rats submitted to surgical laparotomy and anesthetic stress without the induction of I/R; I/R, rats submitted to I/R protocol, consisting of partial ischemia of 70% of the liver, with 30% of hepatic tissue being perfused, by camping of the right hepatic pedicle after laparotomy, for 90 minutes followed by 15 minutes of reperfusion; HBO I/R 1 ATA, rats submitted to 30 minutes of HBO treatment at the pressure of 1 absolute atmosphere (ATA) during the ischemia time of the the experimental I/R protocol. HBO I/R 2 ATA, rats submitted to 30 minutes of HBO treatment at the pressure of 2 ATA during the ischemia time of the experimental I/R protocol. Pre HBO I/R 30', rats submitted to 30 minutes of HBO treatment at the pressure of 2 ATA immediately before the I/R protocol. Pre HBO I/R 90', rats submitted to 90 minutes of HBO treatment at the pressure of 2 ATA immediately before the I/R protocol.

Surgical procedure

Surgical materials were clean and sterile. The animals were anesthetized with xylazine hydrochloride solution (20mg/ml) and ketamine hydrochloride (50mg/ml) in a 1:2 ratio and applied 100mg/Kg. The anesthetic was applied in the peritoneal cavity. The surgical procedure began with medial laparotomy which extended from the lower third of the xiphoid to the pubis. Next, exploration of the abdominal cavity, delicate dissection of the round ligament of the liver and identification of the hepatic pedicle was performed.

Partial hepatic pedicle clamping was carried out using neurosurgery clamps to produce about 70% ischemia of the hepatic parenchyma[1], including the left and median lobes for 90 minutes. The rat was then subjected to 15 minutes of reperfusion. After reperfusion, blood was collected by puncturing the inferior vena cava for enzyme dosage and hepatectomy was performed. The animal sacrifice was performed right after by respiratory and cardiac arrest after diaphragm cutting.

Hyperbaric treatment

The hyperbaric treatment was performed using a collective experimental Hyperbaric Chamber (Sechrist model 2500 B). Oxygen 100% was used in all groups of HBO treatment. The chamber was gradually compressed and decompressed at 1.3 ATA/min for 15 minutes each, which was not considered as treatment time[7,13].

Hepatic function was determined by measuring serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST)[14] 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[15].

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

There was a significant worsening of all the parameters of mitochondrial energy production (state 3, 4, RCR and Swelling) in the I/R group, when compared to the Sham group (I/R <Sham, $p<0.05$) (Figures 1-4). There was also a statistically significant worsening in state 4 (Figure 2), RCR (Figure 3) and mitochondrial edema (Figure 4) in the Pre HBO I/R 90' group compared to the I/R group, whereas the difference was not statistically significant in any other group.

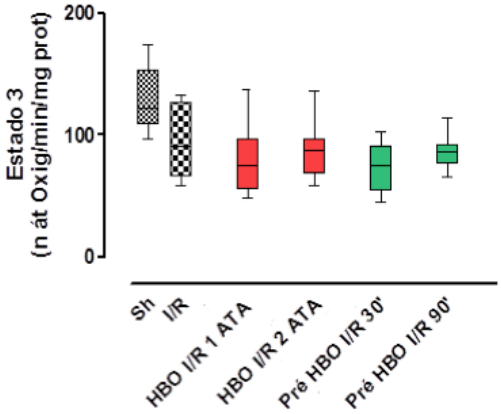

FIGURE 1 - State 3 of mitochondrial respiration chain. The red bars correspond to HBO treatment during ischemia, the green correspond to pre-treatment with HBO, before ischemia. Values were statistically different from Sham in all groups and from I/R in none of the HBO groups.
Sh = rats submitted to surgical stress without ischemia/reperfusion protocol
I/R = rats submitted to surgical ischemia/reperfusion protocol
HBO I/R 1 ATA = rats submitted to HBO treatment with 1 ATA, during 30 minutes while in ischemia
HBO I/R 2 ATA = rats submitted to HBO treatment with 2 ATA, during 30 minutes while in ischemia
Pre HBO I/R 30' = rats submitted to HBO pre-treatment (before ischemia) during 30 minutes
Pre HBO I/R 90' = rats submitted to HBO pre-treatment (before ischemia) during 90 minutes

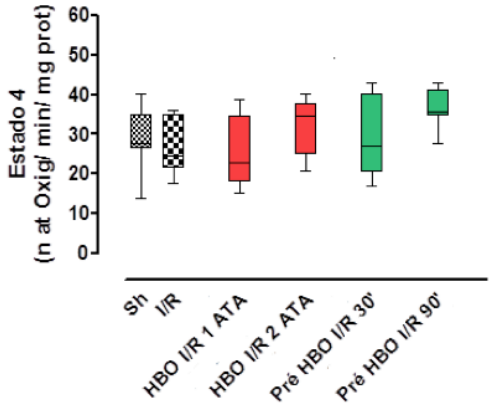

FIGURE 2 - State 4 of mitochondrial respiration chain. The red bars correspond to HBO treatment during ischemia, the green correspond to pre-treatment with HBO, before ischemia. Values were statistically different from I/R in the Pre HBO I/R 90' group.
Sh = rats submitted to surgical stress without ischemia/reperfusion protocol
I/R = rats submitted to surgical ischemia/reperfusion protocol
HBO I/R 1 ATA = rats submitted to HBO treatment with 1 ATA, during 30 minutes while in ischemia
HBO I/R 2 ATA = rats submitted to HBO treatment with 2 ATA, during 30 minutes while in ischemia
Pre HBO I/R 30' = rats submitted to HBO pre-treatment (before ischemia) during 30 minutes
Pre HBO I/R 90' = rats submitted to HBO pre-treatment (before ischemia) during 90 minutes

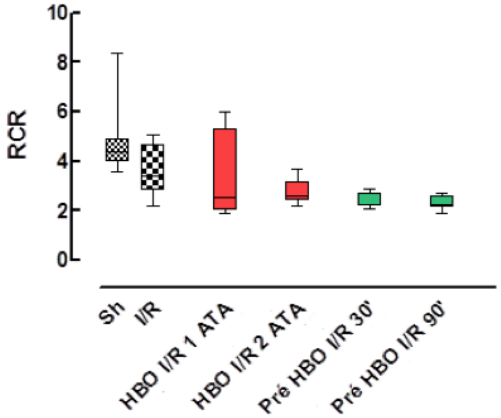

FIGURE 3 - RCR of mitochondria. The red bars correspond to HBO treatment during ischemia, the green correspond to pre-treatment with HBO, before ischemia. Values were statistically different from I/R in the Pre HBO I/R 90' group.
Sh = rats submitted to surgical stress without ischemia/reperfusion protocol
I/R = rats submitted to surgical ischemia/reperfusion protocol
HBO I/R 1 ATA = rats submitted to HBO treatment with 1 ATA, during 30 minutes while in ischemia
HBO I/R 2 ATA = rats submitted to HBO treatment with 2 ATA, during 30 minutes while in ischemia
Pre HBO I/R 30' = rats submitted to HBO pre-treatment (before ischemia) during 30 minutes
Pre HBO I/R 90' = rats submitted to HBO pre-treatment (before ischemia) during 90 minutes

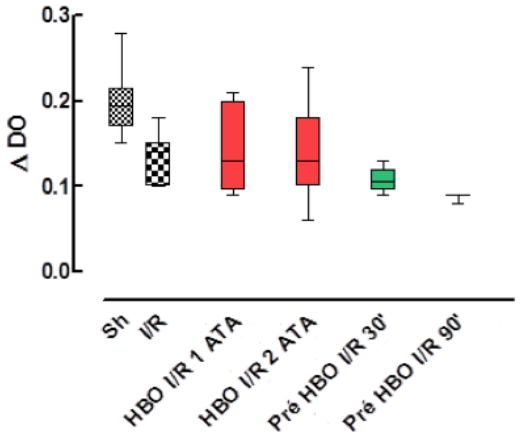

FIGURE 4 - Mitochondrial swelling. The red bars correspond to HBO treatment during ischemia, the green correspond to pre-treatment with HBO, before ischemia. Values were statistically different from I/R in the Pre HBO I/R 90' group.
Sh = rats submitted to surgical stress without ischemia/reperfusion protocol
I/R = rats submitted to surgical ischemia/reperfusion protocol
HBO I/R 1 ATA = rats submitted to HBO treatment with 1 ATA, during 30 minutes while in ischemia
HBO I/R 2 ATA = rats submitted to HBO treatment with 2 ATA, during 30 minutes while in ischemia
Pre HBO I/R 30' = rats submitted to HBO pre-treatment (before ischemia) during 30 minutes
Pre HBO I/R 90' = rats submitted to HBO pre-treatment (before ischemia) during 90 minutes

Hepatic enzyme concentrations were significantly higher in the I/R group compared to the Sham group. Values were also significantly higher when compared to the I/R group in all groups but Pre HBO I/R 30' (Figure 5).

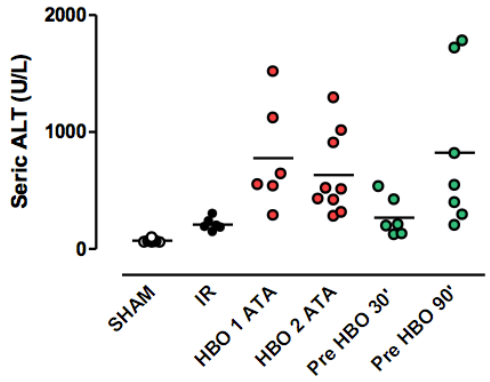

FIGURE 5 - Serum levels of ALT. The red dots correspond to HBO treatment during ischemia, the green correspond to pre-treatment with HBO, before ischemia. Values were statistically different from I/R in all groups, except Pre HBO 30'.
Sh = rats submitted to surgical stress without ischemia/reperfusion protocol
I/R = rats submitted to surgical ischemia/reperfusion protocol
HBO I/R 1 ATA = rats submitted to HBO treatment with 1 ATA, during 30 minutes while in ischemia
HBO I/R 2 ATA = rats submitted to HBO treatment with 2 ATA, during 30 minutes while in ischemia
Pre HBO I/R 30' = rats submitted to HBO pre-treatment (before ischemia) during 30 minutes
Pre HBO I/R 90' = rats submitted to HBO pre-treatment (before ischemia) during 90 minutes

Discussion

For the stage 3 of mitochondrial respiration there was a decrease in the oxygen consumption rate in the I/R group compared with the SHAM group ($p<0.05$) indicating impaired mitochondrial energy production, which indicates a deleterious effect of ischemia/reperfusion in this group. This result is found by other authors[12,13], but in different models of ischemia-reperfusion. Taking into consideration the oxygen consumption in stage 3 it was not found in our study significant difference between the group that underwent ischemia and, reperfusion with or without exposure to HBO, demonstrating that HBO was not harmful when this parameter was evaluated.

Relative the stage 4 of mitochondrial respiration there was no difference between the I/R group and the Sham group. When the groups treated with HBO were compared to the I/R group (not treated), in most of the groups there were no significant differences between values. However, the group Pre HBO I/R 90' showed a significant increase in the state 4 compared to the I/R group, indicating a change in permeability of the mitochondrial inner membrane, responsible for maintaining the proton gradient when the membrane is energized[8]. This indicates that the HBO treatment increased the damage to the mitochondrial membrane, because it took longer for it to return to its basal oxygen consumption rate.

By analyzing the RCR there was not found difference between the SHAM group and the ischemic groups with or without exposure to HBO. Significant difference was found between groups I/R and Pre HBO I/R 90', demonstrating decreased results of RCR in the HBO group. This result suggests that HBO was not effective in accelerating the respiration under energy demand conditions.

The osmotic mitochondrial swelling induced by Ca +2 and phosphate showed a difference between Sham group and all other groups, indicating the I/R process causes damage to the mitochondrial membrane, altering its gradient and changing its permeability, causing edema. There was also a significant increase (decrease in the difference between the previous edema and the induced edema) in mitochondrial swelling when comparing the I/R group and the Pre HBO I/R 90' group, indicating that the HBO treatment in this group caused additional damage to the membrane, increasing the edema.

With regard to serum levels of ALT there was an increase in the results obtained in the I/R group compared to SHAM group ($p<0.05$), which is in agreement with other studies in the literature[5,8,17]. We also found increased levels of ALT in all groups of HBO treatment, but Pre HBO I/R 30', compared to the I/R group. These facts suggest that HBO increased the hepatocellular injury, except for the pre-treatment with 2 ATA for 30 minutes.

There are other studies in this area that show positive results, using other methods of pre conditioning or treatment. For example, the chronic use of HBO treatment showed protective effects[19,20]. Other studies show a beneficial effect not only on mitochondrial function but also in the liver regeneration and protection of the tissue after hours of the ischemic injury[21]. In general, the literature is still controverse, but there are data that suggests a beneficial effect of Hyperbaric Oxygen in the ischemic injury, depending on its duration[22](2. There may be a therapeutic window that is yet to be described, and requires further studies in the area.

Conclusions

The use of hyperbaric oxygen (HBO) before and during ischemia/reperfusion (I/R) did not improve the production of hepatocellular energy reduced by I/R, nor did it prevent the installation of mitochondrial edema induced by I/R. The use of pre-conditioning treatment with HBO for 90 minutes aggravated the mitochondrial damage caused by I/R.

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