Hyperbaric oxygenation improves redox control and reduces mortality in the acute phase of myocardial infarction in a rat model

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Abstract. Among the mechanisms of action of hyperbaric oxygenation (HBO), the chance of reducing injury by interfering with the mechanisms of redox homeostasis in the heart leads to the possibility of extending the period of viability of the myocardium at risk. This would benefit late interventions for reperfusion to the ischemic area. The objective of the present study was to investigate the changes in the redox system associated with HBO therapy maintained during the first hour after coronary occlusion in an acute myocardial infarction (MI) rat model. Surviving male rats (n=105) were randomly assigned to one of three groups: Sham (SH=26), myocardial infarction (MI=45) and infarction+hyperbaric therapy (HBO=34, 1 h at 2.5 atm). After 90 min of coronary occlusion, a sample of the heart was collected for western blot analysis of total protein levels of superoxide dismutase, catalase, peroxiredoxin and 3-nitrotyrosine. Glutathione was measured by enzyme-linked immunosorbent assay (ELISA). The detection of the superoxide radical anion was carried out by oxidation of dihydroethidium analyzed with confocal microscopy. The mortality rate of the MI group was significantly higher than that of the HBO group. No difference was noted in the myocardial infarction size. The oxidized/reduced glutathione ratio and peroxiredoxin were significantly higher in the SH and MI when compared to the HBO group. Superoxide dismutase enzymes and catalase were significantly higher in the HBO group compared to the MI and SH groups. 3-Nitrotyrosine and the superoxide radical were significantly lower in the HBO group compared to these in the MI and SH groups. These data demonstrated that hyperbaric oxygenation therapy decreased mortality by improving redox control in the hearts of rats in the acute phase of myocardial infarction.

Introduction

Advances in the treatment of patients with acute myocardial infarction, through coronary reperfusion, have led to a decrease in hospital mortality rates. However, the injury generated by the reestablishment of blood flow, known as reperfusion injury (RI), still represents a great challenge (1,2). There is strong evidence that reactive oxygen species (ROS) and reactive nitrogen species (RNS), produced during the period of ischemia, trigger an increased production of these reactive species at the time of reperfusion (3-5).

As a way to increase myocardial tolerance to ischemia, approaches such as pre-conditioning and post-conditioning have been proposed; among these are oxidative stress: Hypoxic, ischemic and hyperoxic (3,6). Hyperbaric therapy (HBO) has also been used in different clinical conditions in which ischemia plays a considerable role in tissue injury (7). The Undersea and Hyperbaric Medical Society (North Palm Beach, FL, USA) (8) defines HBO as an intervention in which an individual breathes nearly 100% oxygen, intermittently, inside a hyperbaric chamber that is pressurized to greater than sea level pressure (1 atmosphere absolute, or ATA). The benefits of 100% oxygen therapy administered at pressures of 2.5 ATA result from the increase in the volume of O_2 dissolved in plasma from 0.3% at 1 ATA to more than 3.5% at 2.5 ATA,

resulting in a 3- to 4-fold increase in the diffusion distance of O_2 in tissues (9-11). Thus, improvement in tissue oxygen delivery provided by HBO therapy may limit the evolution of necrosis during the event of myocardial ischemia.

Paradoxically, the increase in O_2, offered by HBO, can intensify oxidative stress due to ROS elevation (12). Notwithstanding, a growing body of evidence suggests that cell signaling mediated by increased production of ROS results in increased activation of antioxidant enzymes, increasing the tolerance of at-risk tissue as has been described with cardiac cells (4,5,13-17).

In our previous study in a rat model, we reported that application of HBO, after induction of acute myocardial infarction (MI), promoted a decrease in the size of the infarct and an increase in rat survival rate (18). In their study of HBO therapy, also in a rat model, Guadalupe *et al* (19) reported higher values of the total antioxidant response and 3-nitrotyrosine in the zone of tissue damage of the left heart, compared to animals not treated with HBO. We identified three other studies in which HBO was used for post-conditioning. Kuhn *et al* (20) used a rat model of coronary occlusion, using systemic embolization, reporting a decrease in the mortality rate of rats treated with HBO, compared to no treatment. Using a dog model, Mogelson *et al* (21) reported on the benefits of therapeutic HBO in improving the outcomes of cardiac infarction. Thomas *et al* (22) compared the outcomes of HBO to rTPA therapy in a dog model of cardiac infarct, concluding that all forms of treatment decrease the severity of injury, with combined HBO therapy and recombinant tissue plasminogen activator (rTPA) treatment providing maximal recovery.

In humans, Yogaratnam *et al* (23) demonstrated that preconditioning of patients with coronary heart disease using HBO, prior to on-pump cardiac surgery, improved ventricular ejection and reduced myocardial injury. Shandling *et al* (24), Stavitsky *et al* (25), Dekleva *et al* (26) and Vlahović *et al* (27) reported the benefits of HBO treatment on cardiac function in patients with thrombolysis who sustained MI. Zhdanov and Sokolov (28) reported that HBO therapy, combined with conventional therapy for MI, effectively liquidated hypoxia and improved the contractile and pumping function of the heart. However, two recent studies (7,12) indicated that the treatment outcomes of HBO therapy for the treatment of patients with MI remains to be fully defined.

The objective of the present study was to investigate the changes in the redox system associated with HBO therapy maintained during the first hour after coronary occlusion in an MI rat model. We analyzed the influence of HBO at the end of the first hour after coronary occlusion, considering that, in the rat, this period is sufficient to cause necrosis of the entire risk area (29,30).

Materials and methods

Animals. Male Wistar rats weighing 250-330 g (11-12 weeks of age) from the Central Animal Facilities of our institution were used. The animals were housed under a 12-h light/dark cycle, at 22-23°C and 54-55% humidity. Rats were fed a pellet rodent diet (Nuvilab CR1, manufactured by Nuvital, Curitiba, Brazil), *ad libitum,* and had free access to water. Surviving male rats (n=105) were randomly assigned to one of three groups: Sham (SH; n=26), Myocardial infarction (MI; n=45) and submitted to Hyperbaric therapy (HBO; n=34). Animals in the SH and MI groups were maintained under oxygenation at ambient pressure for the same time as the treated group.

All procedures were performed according to the principles of ethics of animal experimentation adopted by the Brazilian College of Animal Experimentation (COBEA-www.cobea. org.br), in conformity with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996). The protocol of the present study was approved by the Research Ethics Committee (CEP 0891/09) of the Federal University of São Paulo.

The rats were anesthetized with halothane, intubated and ventilated with a ventilator for rodents (model 683; Harvard Apparatus Co., South Natik, MA, USA). The rats were randomized between the Sham (SH), myocardial infarction (MI) and hyperbaric therapy (HBO) groups. MI was produced in the animals of the MI and HBO groups by ligation of the anterior descending coronary artery, as described previously (31,32). Sham rats underwent a similar procedure, without coronary ligation.

All experiments were initiated at the same time (13:00) in order to avoid influence of the different biological rhythms.

Hyperbaric therapy. Immediately after recovery from anesthesia (approximately 2 min), animals from the HBO group were exposed to 100% O_2 under a pressure of 2.5 atmospheres absolute (ATA) for 60 min. within a hyperbaric chamber for small rodents, as previously described (18). The gas in the chamber was continuously vented to avoid CO_2 retention (<0.1%) and the temperature was maintained in the range of 25-27°C. Compression and decompression were performed at the rate of 0.2 ATA/min (20 kPa/min) and the pressure inside the chamber was stable over a period of 12 to 15 min.

Animals in the SH and MI groups were maintained under oxygenation at ambient pressure for the same time as the treated group. At the end of the protocol, the surviving rats (SH=26, MI=45, and HBO=34). were sacrificed under halothane anesthesia and each heart was removed and sectioned transversely at the middle of the left ventricle (LV). The basal half was used to measure the infarct size, with triphenyltetrazolium (TTZ), according to a previously described protocol (18). The apical half was prepared in gel (OCT-Sakura, Japan) and frozen with liquid nitrogen for cryostat sectioning. The samples were stored at -80°C for further analysis by microscopy.

Western blot analysis. Proteins were extracted as previously described (33). Homogenate protein samples of 30 μg were subjected to SDS-PAGE on 12% polyacrylamide gel. The separated proteins were transferred onto hydrophobic polyvinylidene difluoride membranes (Hybond-P, Amersham Biosciences), and the transfer efficiency was examined with 0.5% Ponceau S. The membranes were soaked in a blocking buffer (5% nonfat dry milk and 0.1% Tween 20 in PBS, pH 7.5) for 1 h at room temperature and then incubated overnight at 4°C with primary antibodies: Mouse monoclonal anti-catalase (dilution 1:1,000, Sigma-Aldrich; Merck KGaA, catalog number C0979), rabbit polyclonal anti-nitrotyrosine (dilution 1:2,000, EMD Millipore, catalog number 06-284), rabbit polyclonal

anti-superoxide dismutase 1 (dilution 1:2,000; Abcam, catalog number ab16831), and rabbit polyclonal anti-peroxiredoxin (dilution 1:2,000, PrxSO$_{2/3}$, Abcam, catalog number ab16830), which recognizes sulfinic (-SO$_2$) and sulfonic (SO$_3$) forms of Prx I to IV; mouse monoclonal anti-GAPDH (dilution 1:2,000, Abcam, catalog number ab8245). After incubation, the membranes were washed three times and then incubated for 1 h at room temperature with horseradish peroxidase-conjugated secondary antibodies (dilution 1:5,000; Abcam, catalog number ab97051). Detection was performed with chemiluminescence reagents (GE Healthcare) and values for target protein were normalized to GAPDH. Dot blot method for nitrotyrosine was analyzed as previously described (34). Samples were transferred onto a nitrocellulose membrane by vacuum for dot blot fluorescence detection performed using Odyssey (LI-COR Biosciences); intensities of dots were determined using Image J 1.43u software (available at http://imagej.nih.gov/ij/, developed by Wayne Rasband, National Institutes of Health).

Determination of intracellular glutathione concentration. Samples were homogenized in 100 mM phosphate buffer (pH 7.0; 250 μl/10 mg tissue) and centrifuged (12,000 x g for 10 min, at 4˚C). To the supernatant (200 μl), 5% of sulphosalicylic acid (200 μl) was added to precipitate the proteins. Detect X® kit (Arbor Assays, Ann Arbor, MI, USA) was then utilized to quantify the total concentration of glutathione (GSH). After incubating the mixture with ThioStar® (Detect X, Arbor Assays) for 15 min, at room temperature, the fluorescence was determined (excitation 390 nm, emission 510 nm), using a spectrophotometer (U-2810; Hitachi), and the reduced GSH concentration measured.

Subsequently, a reaction mixture was added to convert all oxidized glutathione (GSSG) into free GSH, which then reacted with the excess ThioStar® to yield the signal related to the total GSH content. A standard curve was used to calculate the total and reduced glutathione concentrations. The oxidized glutathione concentration was obtained using the following calculation: GSSG=(total GSH-reduced GSH)/2. Detection limits ranged between 38 nM, for free GSH, and 42 nM, for the total GSH. Results were normalized to the muscle fragment weight for between-animal comparisons. All assays were performed in triplicate.

In situ ROS generation. *In situ* microfluorotopography of dihydroethidium (DHE) oxidation products was performed as previously described (35), with 3 μmol/l final DHE concentration. Slides were analyzed by confocal microscopy (Zeiss LSM510) with laser excitation at 488 nm and emission at 610 nm. Controls, performed by incubating slides for 30 min with Peg-SOD (500 U/ml), indicated preferential detection of superoxide with DHE. Quantitative analysis of fluorescence images was performed with Leica Qwin Plus (Leica Microsystems Ltd., Switzerland) software.

Quantification of the by-products of nitric oxide. Myocardial homogenates were prepared under liquid N$_2$. After centrifugation (13,400 x g for 20 min, at 4˚C), 20 μl aliquots were injected into NOA (Nitric Oxide Analyzer model 280; Sievers Instruments, USA), with VCl$_3$ and HCl (at 95˚C) used as reductants, as previously described (36). Nitric oxide (•NO),

nitrite (NO$_2^-$) and nitrate (NO$_3^-$) by-products were normalized for protein concentration.

Statistical analysis. The data are expressed as mean ± SEM. The Student t-test was used for comparisons of infarct sizes and the Chi-square test was used to compare mortality. Two-way ANOVA was applied to parametric data using Newman-Keuls to identify statistical differences. Kruskal-Wallis was performed on non-parametric data, associated with the Dunn's test to identify statistical differences. The statistical program used was GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA, USA). Differences with P≤0.05 were considered significant.

Results

Mortality and myocardial infarction size. Immediate mortality was established when it occurred in the period between the introduction of the animal into the HBO chamber until the end of the decompression (~90 min). In the MI group, 27 animals died during this period, whereas HBO therapy was effective in improving post-infarction survival, with only 6 immediate deaths noted. Fig. 1 shows the percentage of mortality for all animals submitted to the protocol, showing a clearly lower rate of mortality in the HBO group (15%) than that in the MI group (37.5%). Additionally, no significant difference in infarct size was observed (Fig. 1B) between the experimental groups (MI 38±2.0% vs. HBO 43±2.5%).

Intracellular glutathione concentration. In order to investigate whether redox processes are involved in the protective effect induced by HBO therapy, we first addressed the redox status in LV homogenates, by measuring the amount of reduced and oxidized glutathione. The infarcted groups showed (Fig. 1C) a reduction in the GSH redox buffer (MI, 97±11 and HBO, 68±5 μM/mg) when compared to the SH group (126±10 μM/mg). However, disulfide (Fig. 1D) was significantly increased only in the HBO group (15±1 μg/mg) when compared to that in the SH (9±1 μM/mg) and MI (12±1 μM/mg) groups. The GSH/GSSG ratio showed that the values in the HBO group (10±1) underwent a greater alteration of this intracellular redox buffer, when compared to those of SH (30±4) and MI (17±3) groups (Fig. 1E). There was no statistical significance in the comparison of SH and MI groups (Fig. 1E). Therefore, these results indicate that myocardial infarct decreases the amount of reduced glutathione and HBO therapy accentuates such a pro-oxidizing effect.

ROS generation. The higher oxidative environment promoted by MI may be caused by increased oxidant generation or by reduced antioxidant defense. First, we assessed the potential to generate ROS by addressing the total amount of DHE-oxidation by-products by microfluorotopography on slides of the myocardium in the SH group (Fig. 2A), the MI group (Fig. 2B), the HBO group (Fig. 2C) and the MI group (Fig. 2D). The fluorescence of DHE-oxidation products, measured in units/area, were significantly lower in the HBO group (0.86±0.08) compared to the MI group (1.87±0.08) and the SH group (1.40±0.11) (Fig. 2E). Although we could not exclude the potential interference by other
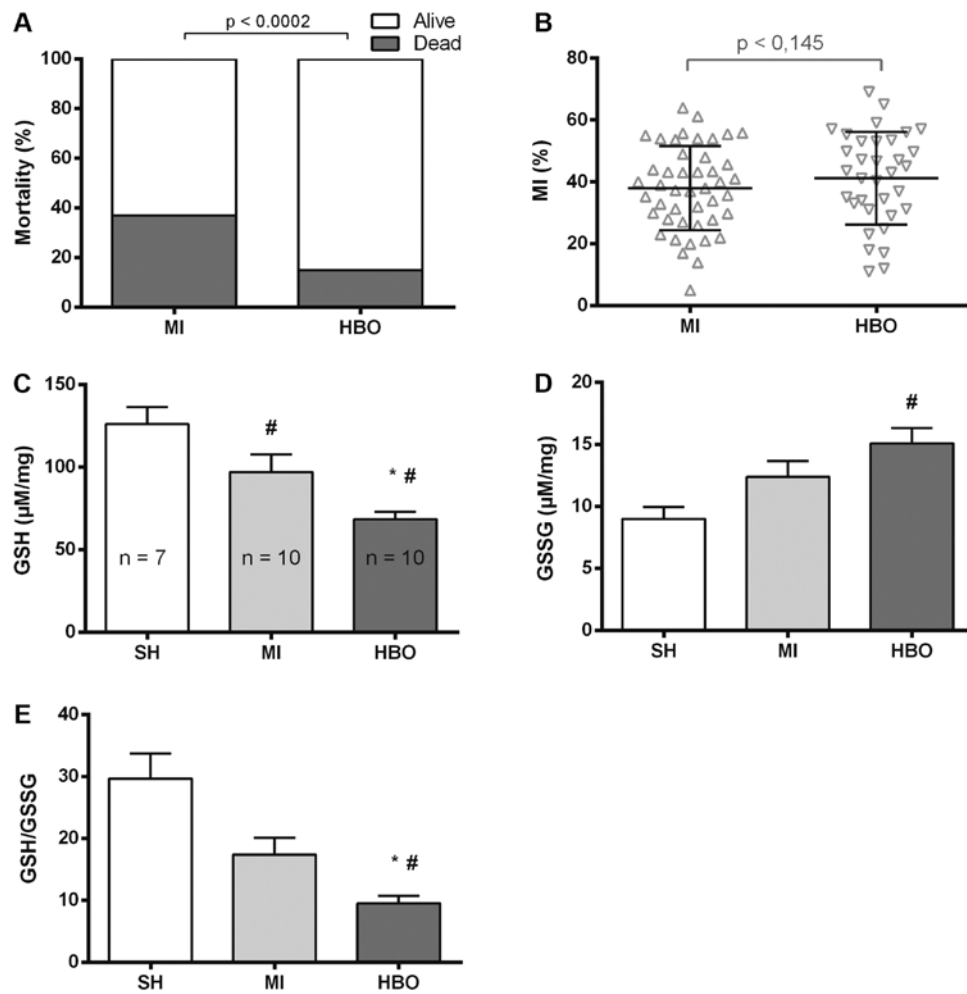
Figure 1. (A) Mortality rate, as expressed as percentages of infarcted animals, without therapy (MI) and infarcted animals submitted to hyperbaric oxygenation (HBO). (B) MI size as expressed as a percentage of the LV perimeter. (C) GSH values. (D) GSSG values. (E) GSH/GSSG ratio. The oxidized glutathione concentration was obtained using the following calculation: GSSG=(total GSH-reduced GSH)/2. *P<0.05 in relation to the MI group, #P<0.05 in relation to the SH group. Groups: SH, Sham; MI, myocardial infarction; HBO, hyperbaric therapy; GSH, glutathione; GSSG, glutatione disulfide; LV, left ventricle.
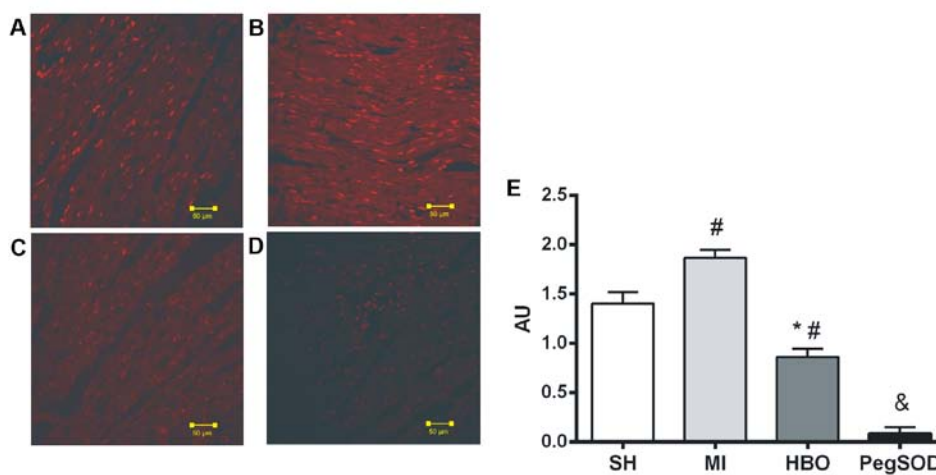

Figure 2. Microfluorotopography of dihydroethidium (DHE) oxidation products, on slides of the myocardium in the (A) SH group, (B) MI group, (C) HBO group and (D) MI group. The slides were incubated with 500 U/ml Peg-SOD. (E) The total fluorescence intensities of the studied groups. *P<0.05 relative to the MI group; #P<0.05 relative to the SH group, &P<0.05 between the SH, MI, and HBO. Groups: SH, Sham; MI, myocardial infarction; HBO, hyperbaric therapy.

peroxides (37), the pre-incubation with pegylated SOD was able to inhibit the oxidation of DHE (0.09±0.06), showing

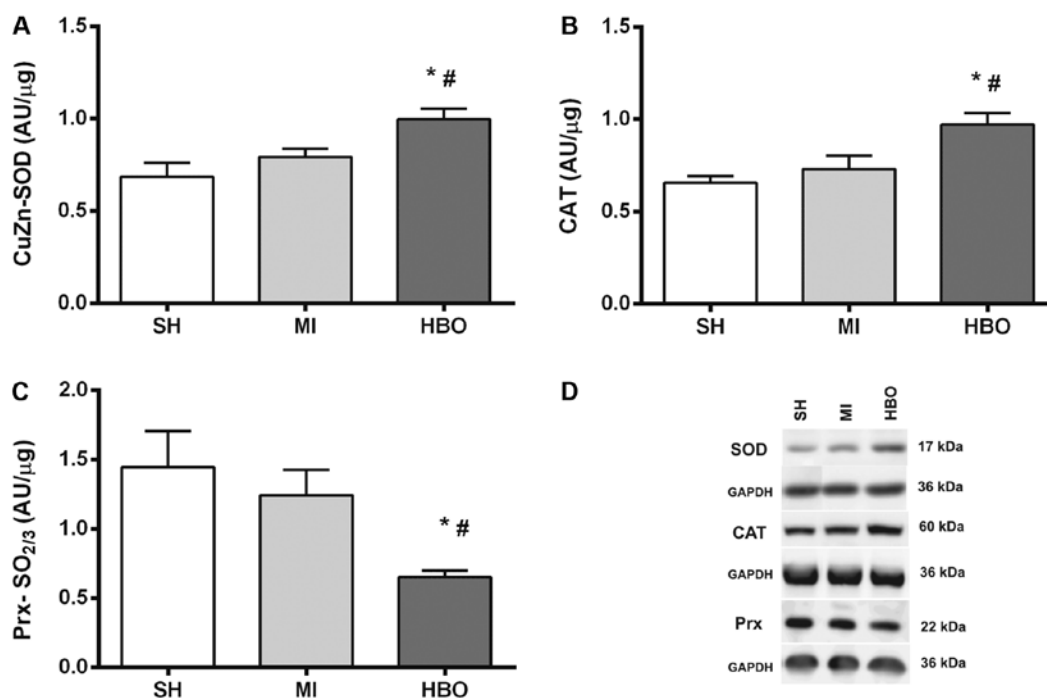greater specificity of the technique to the superoxide anion. Thus, hyperbaric therapy was able not only to reduce the

Figure 3. Values (mean ± SEM) for (A) SOD expression, (B) CAT expression and (C) Prx-SO$_{2/3}$ expression in the SH, MI, and HBO groups. (D) Immunoblots. *P<0.05 in relation to MI, #P<0.05 in relation to SH. Groups: SH, Sham; MI, myocardial infarction; HBO, hyperbaric therapy; SOD, superoxide dismutase; CAT, catalase; Prx, peroxiredoxin.

production of superoxide induced by infarction, but also to minimize myocardial levels in relation to non-infarcted animals (Fig. 2E).

Results of western blot analysis of SOD, catalase and peroxiredoxin. Due to the higher levels of superoxide promoted by MI, combined with the protective effect induced by HBO, the antioxidant capacity was investigated by assessing the expression of SOD, whose values showed that HBO therapy significantly increased enzyme expression in the HBO group (1.0±0.06 AU/μg) relative to the MI (0.79±0.04 AU/μg) and SH (0.69±0.08 AU/μg) groups, with no significant difference between the SH and MI groups (Fig. 3A). In addition, expression of catalase (CAT) was investigated due to the intriguing effect observed in the HBO group concerning the higher levels of oxidized glutathione. Similarly to that observed with the SOD enzyme, HBO therapy significantly increased CAT enzyme expression in the HBO group (0.97±0.06 AU/μg) in relation to the MI (0.73±0.07 AU/μg) and SH (0.66±0.04 AU/μg) groups. There was no significant difference between the SH and MI groups (Fig. 3B). Thus, HBO therapy was able to upregulate enzymatic antioxidants. In order to better investigate the antioxidant activity, the expression of peroxiredoxin (Prx) hyperoxidation was assessed. Importantly, the expression of the sulfonylated form of Prx (SO$_{2/3}$) protein was lower in the HBO group (0.65±0.05 AU/μg), when compared to MI (1.24±0.18 AU/μg) and SH (1.45±0.26 AU/μg) values, corroborating with better redox control. There was no significant difference between the MI group and the SH group (Fig. 3C). Therefore, not only the total expression of antioxidants was increased such as SOD and CAT, but also the Prx hyperoxidation levels were decreased by HBO therapy.

Nitric oxide by-products and 3-nitrotyrosine. Nitric oxide and its oxidation-derived by-products show a dual role during normal and pathological conditions, including MI (38,39). Coronary occlusion caused a significant increase in nitrate (NO$_3^-$) (nM/mg protein) in the MI (1.37±0.26) and HBO (1.58±0.41) groups in relation to the SH group (0.56±0.08). No significant difference was found between the two groups with MI (Fig. 4A), which represents a greater availability of nitric oxide in an environment with increased oxidative stress. No significant difference was found in levels of nitrite (NO$_2^-$) (nM/mg protein) between the SH (0.51±0.08), MI (0.69±0.13) and HBO (0.74±0.20) groups (Fig. 4B). In addition, the positive labeling of 3-nitrotyrosine (AU/μg prot) by dot blot analysis (Fig. 4C) showed a difference between groups. A lower amount of nitrated protein was found in the HBO group (2.40±0.18) compared to the MI (3.08±0.16) and SH (3.36±0.20) groups. There was no significant difference between the SH and MI groups. Therefore, these data suggest that MI induces increased production of NO while HBO is effective to prevent such NO-related modification in global target proteins, as reflected by the lower levels of 3-nitrotyrosine.

Discussion

In the present study, the mortality rate of the animals in the hyperbaric oxygenation (HBO) group was significantly lower than that of the animals in the myocardial infarction (MI) group, indicating a favorable action of hyperbaric oxygenation in survival. This result is in accordance with a recent review of the literature published by Bennett *et al* (7). These authors related the reduced mortality by HBO in MI to the limitation of infarcted muscle mass and decreasing cardiac arrhythmias. As in our animals there was no difference noted in MI size, the reduced mortality rate of the HBO animals should be linked to
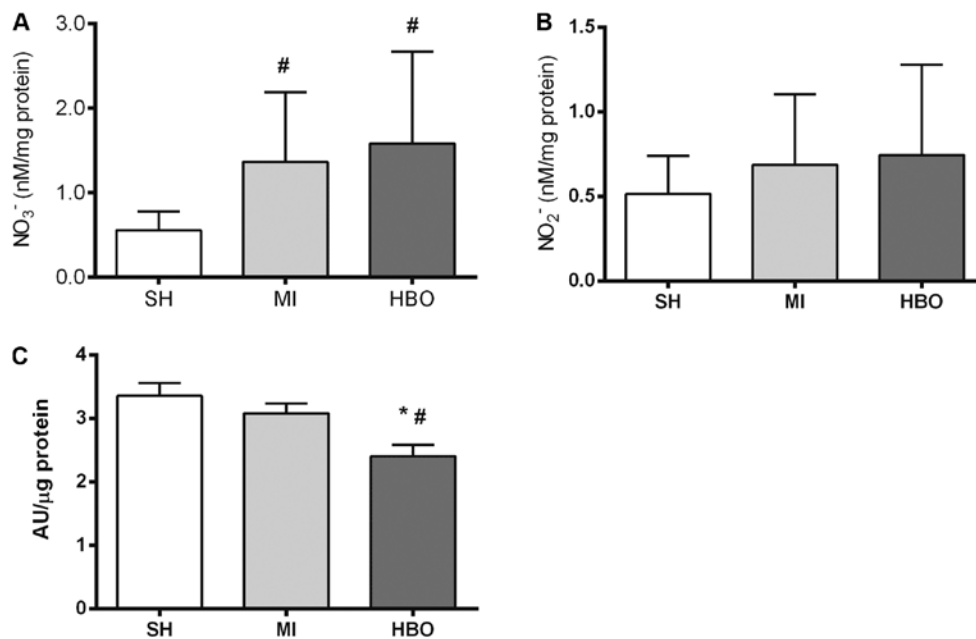
Figure 4. Graphs showing values (mean ± SEM) for (A) NO_3^-, (B) NO_2^- and (C) nitrated proteins. *P<0.05 in relation to MI, #P<0.05 in relation to SH. Groups: SH, Sham; MI, myocardial infarction; HBO, hyperbaric therapy.

the beneficial effect on the alteration of infarct-induced redox processes, which are important for maintaining a favorable environment for cell survival and heart rhythm (40,41).

Our results indicate that therapy with 2.5 ATA of HBO resulted in similar mean infarct sizes in the MI and HBO group. In a previous study by our group (18), we reported that hyperbaric treatment reduced MI size and a lower mortality rate was not observed. The discrepancy between the present data with data of our previous study may result from differences in the protocols employed. In the present protocol, the control of temperature (~25°C) and the longer handling time in our protocol, due to the collection and preparation of samples taken after treatment with HBO, are crucial factors in the enzymatic action and may be responsible for the verified differences.

The lower level found in the GSH/GSSG ratio in the HBO group suggests an increase in glutathione (GSH) use, indicating a higher efficiency of the antioxidant mechanisms to protect against oxidative stress present in these animals. The same response was not observed in the untreated animals of the MI group. The higher glutathione disulfide (GSSG) formation indicates the increased levels of reactive oxygen species (ROS) formation induced by MI.

Importantly, the improved buffering capacity of antioxidants promoted by HBO therapy is supported by the higher amount of superoxide dismutase (SOD) and catalase (CAT), which resulted in reduced levels of oxidant generation and their post-translational modifications, such as ROS and 3-nitrotyrosine, respectively. Moreover, the lower levels of Prx-$SO_{2/3}$ strengthened such a protective effect. Collectively, the pro-survival effect of HBO, may be promoted by a hormetic-like effect (40,42). The acute exposure to the higher oxidation conditions was able to affect a major ROS buffering system, triggering a global response that prevented the more accentuated disruption of the redox processes. These factors resulted in a more prepared system to counteract impairments of the myocardial milieu.

The lower levels of ROS indicated by DHE oxidation may have resulted from increased SOD and CAT expression. Although the mechanisms by which HBO therapy induces an antioxidant response remain unclear, it has been reported to involve a Nrf2-dependent effect (43,44) triggered in a pro-oxidizing environment (31). Indeed, we observed that HBO decreased the GSH/GSSG ratio, confirming such Nrf2-inducing conditions. Moreover, due to the fast induction of SOD and CAT reported here, we cannot exclude potential regulation by protein degradation systems, as both enzymes were reported to be targeted by proteasome degradation (45,46). Thus, further studies are necessary to better understand the mechanism by which acute HBO induces an antioxidant response.

The transient sulfinic acid peroxiredoxin (Prx) intermediate has been attributed to control redox signaling. Such reversibility, which is consistent with signaling events, is lost by its hyperoxidation to sulfinic and sulfonic irreversible forms (47). Importantly, HBO decreased the amount of Prx-$SO_{2/3}$H providing additional evidence that the normal redox signaling is favored by acute hyperoxic treatment. It is thought that the intensity of Prx I/II hyperoxidation modulates ROS, which regulates signal transduction pathways for cell apoptosis, survival or repair of damaged proteins (12,48). Therefore, accumulation of the inactive form of Prx (Cyx-$SO_{2/3}$H) enzyme may lead to an even greater increase in cellular H_2O_2 levels, resulting in increased apoptosis, as shown in cells that do not express sulfiredoxin (49). Surprisingly, the non-infarcted group showed higher levels of Prx-$SO_{2/3}$H, which may reflect a temporal Prx inactivation to sustain ROS-mediated regulation promoted by stress (50), such as associated with sham surgical intervention. Further studies may clarify the effect of these results on apoptosis in the later phase of MI.

Regarding reactive nitrogen species, we observed lower values of 3-nitrotyrosine in the animals of the HBO group in relation to the SH and MI groups, indicating lower nitration

of proteins by peroxynitrite resulting from the interaction of NO with $\bullet O_2^-$ (51). NO production under physiological conditions occurs via the L-arginine/NO synthase pathway, which is O_2-dependent (38). Thus, the hypoxia and/or ischemia present in the MI end up compromising NO production by this route. Nitrate and nitrite, which are NO oxidation products, can be recycled *in vivo* to form NO again, representing an important alternative source of NO under physiological and pathological conditions (38). NO and its by-products have been identified as important signaling agents, regulating mitochondrial respiration and ROS production (39,52). Since the nitrate levels in the MI and HBO groups were not different, the most marked nitration in the MI animals was probably due to the higher $\bullet O_2^-$ levels, allowing a greater formation of peroxynitrite. It is important to note that the higher level of nitrate found in the HBO group, when associated with less nitration, shows greater NO bioavailability in this group.

One limitation of the present study must be mentioned. The aim of assessing the acute effects of HBO following coronary occlusion restricted our interests solely to the immediate effects of the treatment. The effects of HBO on the evolution of MI in the long term, remains to be studied.

In conclusion, our data showed that HBO animals had greater ability to control pro-oxidants and antioxidants during myocardial ischemia. This action should be the reason for the reduced mortality rate in the treated rats.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MO, EA, LB and LS contributed to the design of the study, acquisition of data, and result analyses. AS performed statistical analyses and LT introduced a new research technique, analyzed the data and revised the manuscript for important intellectual content. JK, FL and PT raised grant funding, contributed to the design of the study, and revised the manuscript. All the authors read and approved the final manuscript. All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

Ethics approval and consent to participate

All procedures were performed according to the principles of ethics of animal experimentation adopted by the Brazilian College of Animal Experimentation (COBEA-www.cobea. org.br), in conformity with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996). Our protocol was approved by the Research Ethics Committee (CEP 0891/09) of the Federal University of São Paulo.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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