ORIGINAL ARTICLE

UDC: 616.94-085.835-092.9
DOI: https://doi.org/10.2298/VSP191026142J

The effect of hyperbaric oxygenation on cardiodynamics and oxidative stress in rats with sepsis

Efekti hiperbarične oksigenacije na kardiodinamiku i oksidacioni stres kod pacova sa sepsom

Aleksandar Jevtić*, Vladimir Živković†, Milica Milinković†, Željko Mijailović‡, Nevena Draginić‡, Marijana Andjić‡, Andjela Milojević Šamanović§, Sergey Bolevich‖, Vladimir Jakovljević†‖

***Institute for Orthopedic Surgery "Banjica", Belgrade, Serbia; University of Kragujevac, Faculty of Medical Sciences, †Department of Physiology, ‡Department of Pharmacy, §Department of Dentistry, Kragujevac, Serbia; ‖1st Moscow State Medical University IM Sechenov, Department of Human Pathology, Moscow, Russian Federation**

Abstract

Background/Aim. Dysfunctions at the cellular, tissue, and organ level, which can result in death, are caused by metabolic changes and affection on the regulation of gene transcription and micro- and macrocirculation. The aim of the present study was to assess the impact of hyperbaric oxygenation (HBO) on isolated heart as well as on the oxidative status of rats with sepsis. **Methods.** The investigation included male Wistar albino rats classified into three groups: the first group was a control group (CTRL); the second group included animals exposed only to the induction of sepsis without HBO treatment (the Sepsis group), while the third group included animals treated with HBO after the induction of sepsis (the Sepsis + HBO group). For the induction of sepsis, fecal peritonitis model was used (3 mL/kg of fecal suspension administered intraperitoneally). After the induction of sepsis, the rats were exposed twice a day (on 12 hours) to HBO treatment at 2.8 atmospheres absolute (ATA) for 90 minutes over a period of 3 days. 72 h after the confirmation of sepsis, the animals were sacrificed and the hearts were retrogradely perfused on the Langendorff apparatus at a gradually increased coronary perfusion pressure (CPP = 40–120 cm H_2O). The following parameters of heart function were continuously recorded: maximum and minimum rate of left ventricular pressure development (dp/dt max, dp/dt min); systolic and diastolic left ventricular pressure (SLVP and DLVP); heart rate (HR). Coronary flow (CF) was measured flowmetrically. Following oxidative stress markers were measured: nitrites (NO_2^-), superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), index of lipid peroxidation (TBARS), activity of superoxide dismutase (SOD) and catalase (CAT) and the level of reduced glutathione (GSH). **Results.** There were no significant differences in dp/dt max, dp/dt min, SLVP and HR between the groups. CF was statistically significantly higher ($p < 0.01$) in the sepsis group. The values of all cardiac oxidative markers were lower in the sepsis + HBO group ($p < 0.05$), while systemic pro-oxidative and antioxidative parameters were unchanged. **Conclusion.** Our results showed that HBO treatment was not associated with improved cardiac function and coronary perfusion, while expressed promising beneficial effects on cardiac oxidative stress.

Key words:
hyperbaric oxygenation; oxidative stress; sepsis; heart; rats.

Apstrakt

Uvod/Cilj. Disfunkcija na nivou ćelije, tkiva ili organa, koja može imati za posledicu smrtni ishod, javlja se usled metaboličkih promena i poremećaja regulacije transkripcije gena i mikro- i makro cirkulacije. Cilj ove studije bio je da se proceni uticaj hiperbarične oksigenacije (HBO) na izolovano srce, kao i na oksidacioni status pacova sa sepsom. **Metode.** Istraživanjem su obuhvaćeni mužjaci Wistar albino pacova klasifikovani u tri grupe: prva grupa je bila kontrolna grupa (CTRL), drugu grupu su činile životinje izložene samo sepsi bez HBO tretmana (grupa Sepsa), dok su u trećoj grupi životinje tretirane HBO nakon indukcije sepse (grupa Sepsa + HBO). Za indukciju sepse korišćen je model fekalnog peritonitisa (3 mL/kg fekalne suspenzije, intraperitonealno). Posle indukcije sepse, pacovi su bili izloženi dva puta dnevno (tokom 12 sati) HBO tretmanu sa 2,8 apsolutnih atmosfera (ATA) tokom 90 minuta u periodu od 3 dana. 72 h nakon potvrđivanja sepse, životinje su žrtvovane, a srca su retrogradno perfundovana na Langendorfovom aparatu, pri postepenom povećanju koronarnog perfuzionog pritiska (CPP = 40–120 cm H_2O). Sledeći par

Correspondence to: Vladimir Živković, University of Kragujevac, Faculty of Medical Sciences, Department of Physiology, Svetozara Markovića 69, 34 000 Kragujevac, Serbia. E-mail: vladimirziv@gmail.com

ametri srčane funkcije su kontinuirano mereni: maksimalna i minimalna stopa promene pritiska u levoj komori (dp/dt max, dp/dt min); sistolni i dijastolni pritisak leve komore (SLVP i DLVP) i srčana frekvenca (HR). Koronarni protok (CF) je meren floumetrijski. Određivani su sledeći markeri oksidacionog stresa: nitriti (NO_2^-), superoksid anjon radikal (O_2^-), vodonik peroksid (H_2O_2), indeks lipidne peroksidacije (TBARS), aktivnost superoksid dismutaze (SOD) i katalaze (CAT) i nivo redukovanog glutationa (GSH). **Rezultati.** Nije bilo značajne razlike u dp/dt max, dp/dt min, SLVP i HR između grupa. CF je bio statistički značajno veći ($p < 0,01$) u grupi sa sepsom. Vrednosti svih srčanih oksidacionih markera bile su niže u grupi sepsa + HBO ($p < 0,05$), dok su sistemski pro-oksidacioni i antioksidacioni parametri bili nepromenjeni. **Zaključak.** Naši rezultati su pokazali da HBO tretman nije bio povezan sa poboljšanom funkcijom srca i koronarnom perfuzijom, dok je ostvario obećavajući korisne efekte na oksidacioni status u srcu pacova.

Ključne reči:
hiperbarička oksigenacija; stres, oksidativni; sepsa; srce; pacovi.

Introduction

A life-threatening organ dysfunction caused by a disrupted response of the host organism to infection is sepsis, a condition whose global epidemiological significance is difficult to assess, and which places a heavy financial burden on health systems. Dysfunctions at the cellular, tissue, and organ level, which can result in death, are caused by metabolic changes and affection on the regulation of gene transcription and micro- and macrocirculation [1–3]. Much progress has been made in the knowledge of the pathogenetic mechanisms mentioned above in the last few decades, but extensive trials are still needed to implement newer modalities of therapy into sepsis treatment.

In addition to immune and metabolic disorders, cardiovascular disorders develop. The two manifestations of cardiac dysfunction are the hyperdynamic (warm shock) and the hypodynamic (cold shock) phase [4, 5]. The hyperdynamic phase is characterized by the elevated vascular tone and low cardiac output, while in the hypodynamic phase we have a reverse situation – decreased vascular tone and increased cardiac output, and a difference in the form of manifestation in adults and the pediatric population was observed [5]. Tests on the isolated cardiomyocyte revealed that with the passage of time from the onset of sepsis, an increase in heart rate was observed, as well as a decrease in the maximum and minimum rate of left ventricular pressure development and a decrease in diastolic pressure in the left ventricle [5–7]. The basis of these changes is the production of cytokines, whose indirect influence is reflected by the increased production of vasoactive mediators [5]. The association of Ca^{2+} receptors, which control Na^+/K^+-ATPase function in cardiomyocytes, with the onset of cardiac dysfunction in sepsis in an animal model has also been demonstrated [4].

Hyperbaric oxygenation (HBO) is the process of exposing the whole organism to 100% oxygen at elevated pressure [greater than 1 atmosphere absolute – ATA (760 mmHg)] at different time intervals. The effect of this treatment, whose effects can be divided into primary and secondary, is based on the principles of Henry and Dalton's law. The primary effect is the percentage increase in dissolved oxygen in the circulation, while the secondary effects are vasoconstriction, neovascularization, and a decrease in gas volume [8–10]. Vasoconstriction leads to an increase in vascular resistance and arterial pressure, and an increase in pressure in the left ventricle, an increase in the maximum and minimum rate of left ventricular pressure development, bradycardia and a decrease in cardiac output [11, 12]. The mechanisms underlying this cardiovascular response are linked to baroreceptor-mediated regulation [11].

HBO is a potential intervention for the prevention of septic shock and can also be used in the treatment of severe pancreatitis, diabetic foot ulcer, carbon monoxide poisoning and other conditions [13]. Studies addressing the use of HBO in the treatment of sepsis have primarily addressed the benefits of HBO in the domain of cytokine production, especially about the increased expression of interleukin (IL)-10 and the decreased levels of IL-6 [13]. More importantly, due to its mechanism of action, HBO can affect the production of oxidative stress biomarkers and thus change redox homeostasis in sepsis, which could be responsible for the potential positive impact of this procedure [14].

Having in mind that there are almost no studies that investigate the effects of hyperbaric oxygenation on cardiac function and coronary circulation during sepsis, we aimed to assess the potential impact of this therapeutic approach on isolated heart as well as on cardiac and systemic oxidative status of rats with sepsis.

Methods

Animals and study design

The study was carried out on eighteen male Wistar albino rats, 8 weeks old, body weight 200 ± 30 g. The animals were kept in an artificial 12-h light–dark cycle (8:00 a.m–8:00 p.m.) at room temperature (22 ± 1°C). Water and food were available *ad libitum*. The animals were housed in their respective groups in a collective cage and received water and standard laboratory chow. The animals were classified into three groups.

The first group (n = 6) was the control group (CTRL). The second group (n = 6) included animals exposed only to the induction of sepsis without HBO treatment (the Sepsis group), while the third group (n = 6) included animals treated with HBO after the induction of sepsis (the Sepsis + HBO group).

Ethical standards

This research was carried out in the Laboratory for Cardiovascular Physiology of the Faculty of Medical Sciences, University of Kragujevac, Serbia. The study protocol was ap-

proved by the Ethical Committee for the welfare of experimental animals of the Faculty of Medical Sciences, University of Kragujevac, Serbia. All experiments were performed according to EU Directive for the welfare of laboratory animals (86/609/EEC) and the principles of Good Laboratory Practice.

Sepsis induction protocol

Firstly, fresh feces were collected from a heterogeneous group of rats. The feces were then dissolved in saline and this mixture was homogenized and filtered through gauze to obtain fecal suspension. Animals were anesthetized by intraperitoneal administration of ketamine and xylazine (10 mg/kg, 5 mg/kg, respectively) and 3 mL/kg of fecal suspension was administered intraperitoneally. For the confirmation of sepsis, the previously established clinical rat scoring system (Table 1) was used for each animal as well as rectal body temperature and biochemical indicators of sepsis [C reactive protein (CRP) and procalcitonin (PCT) values]. Animals from both groups were monitored for the next 72 hours before sacrifice [15].

Table 1

Clinical rat scoring system for the confirmation of sepsis and the severity of sepsis

Characteristic	Scoring range
Hunched	0–1
Bloated	0–1
Conjunctival injection/mucky eyes	0–1
Piloerection	0–1
Lack of movement	0–2
Lack of alertness	0–2

Legend for scoring: absence (0), presence (1) or marked presence (2).
The total score of 0 to 3 denotes mild sepsis and ≥ 4 severe sepsis.

HBO treatment protocol

HBO treatment was carried out in a specially constructed hyperbaric chamber for rats (HYB-C 300). After the induction of sepsis, the rats were exposed twice a day (on 12 hours) to 100% O_2 at 2.8 ATA for 90 minutes for 3 days. To avoid the effects of diurnal rhythm variation, the HBO session started at the same time, each day [16].

Ex vivo assessment of heart function

72h after the confirmation of sepsis, animals from all groups were anesthetized with short-term narcosis induced by intraperitoneal application of ketamine (10 mg/kg) and xylazine (5 mg/kg) and sacrificed by decapitation. The hearts were then rapidly isolated and retrogradely perfused on Langendorff apparatus (Langendorff apparatus, Experimetria Ltd, 1062 Budapest, Hungary) through the ascending aorta at a gradually increased coronary perfusion pressure (CPP = 40–120 cm H_2O). The hearts were perfused with Krebs–Henseleit solution, while a transducer was inserted in the left ventricle to continuously record the following parameters of myocardial function: maximum and minimum rate of left ventricular pressure development (dp/dt max, dp/dt min), systolic and diastolic left ventricular pressure (SLVP and DLVP), and heart rate (HR). Coronary flow (CF) was measured flowmetrically. The perfusion started at CPP = 70 cm H_2O and hearts were allowed to equilibrate until HR and contractility reached steady-state. After stabilization (approximately 30 min), the CPP was gradually increased from 40, 60, 80, 100 and 120 cm H_2O to estimate coronary autoregulation. At each value of CPP, the coronary venous effluent was collected for the determination of oxidative stress parameters.

Evaluation of systemic oxidative stress

At the moment of sacrificing animals, blood samples were collected from jugular vein in order to estimate following pro-oxidants in plasma: nitrites (NO_2^-), superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), index of lipid peroxidation (thiobarbituric acid reactive substances, TBARS) and parameters of antioxidative defense system in erythrocytes samples: activity of superoxide dismutase (SOD and catalase (CAT) and the level of reduced glutathione (GSH). All parameters were measured on the spectrophotometer apparatus (Shimadzu UV 1800, Japan).

Evaluation of cardiac oxidative stress

The coronary venous effluent from each value of CPP (40–120 cm H_2O) was collected for the determination of oxidative stress parameters from the isolated rat heart. The following oxidative stress parameters were determined spectrophotometrically: NO_2^-, O_2^-, H_2O_2 and TBARS.

Markers of oxidative stress

Nitric oxide decomposes rapidly to form stable metabolite nitrite/nitrate products. NO_2^- level was measured spectrophotometrically at a wavelength of 543 nm and used as an index of nitric oxide (NO) production using the Griess reagent as previously described by Green et al. [17]. The measurement of H_2O_2 is based on oxidation of Phenol Red by hydrogen peroxide, in a reaction catalyzed by horseradish peroxidase (HRPO) at 610 nm, as previously described by Pick and Keisari [18]. The index of lipid peroxidation was estimated by measuring of TBARS using 1% thiobarbituric acid (TBA) in 0.05 NaOH incubated with the plasma as previously described [19]. The concentration of O_2^- was measured by Nitro Blue Tetrazolium (NBT) reaction in hydroxymethylaminomethane (TRIS) buffer with a plasma sample at 530 nm as previously described by Auclair and Voisin [20]. The whole analysis was determined using the spectrophotometrical

method (UV-1800 UV – Vis Spectrophotometer by Shimadzu Scientific Instruments Inc).

Antioxidative enzymes

For the determination of antioxidant parameters, isolated erythrocytes were prepared according to McCord and Fridovich [21]. SOD activity was determined by the epinephrine method described by Misra and Fridovich [22]. A 100 μL lysate and 1 mL carbonate buffer were mixed, and then 100 μL of epinephrine was added. Detection was performed at 470 nm. CAT activity was determined according to Beutler [23]. Lysates were diluted with distilled water (1:7 v/v) and treated with chloroform-ethanol (0.6:1 v/v) to remove hemoglobin and then, 50 μL CAT buffer, 100 μL sample, and 1 mL 10 mM H_2O_2 were added to the samples. Detection was performed at 360 nm. The level of reduced glutathione (GSH) was determined based on GSH oxidation with 5.5-dithio-bis-6.2-nitrobenzoic acid, as previously described by Beutler [24]. Measuring was performed at 420 nm.

Determination of CRP and PCT values

Serum CRP and PCT were detected using specific enzyme-linked immunoassay kits according to the manufacturer's instructions [25].

The measurement of rectal temperature

The rectum temperature was continuously monitored using a digital thermometer (PIC solutions, Artsana S.p.A., Grandate, Italy).

Drugs

All kits, reagents and substances used in the study were purchased from Sigma-Aldrich Chemie GmbH Eschenstrasse 5, 82024 Taufkirchen, Germany.

Statistical analysis

IBM SPSS Statistics 20.0 Desktop for Windows was used for statistical analysis. The distribution of data was checked by the Shapiro-Wilk test. Where distribution between groups was normal, statistical comparisons were performed using the one-way analysis of variance (ANOVA) tests with a Tukey's *post hoc* test for multiple comparisons. Kruskal-Wallis test was used for the comparison between groups when the distribution of data was different from normal. Values of $p < 0.05$ were considered to be statistically significant.

Results

Confirmation of sepsis and scoring of sepsis severity

The distribution of the septic score was presented in Table 2. Of the septic animals surviving to sacrifice (n = 12), 22% were scored mild and 78% severe sepsis (Table 2). In addition, a whole group of animal's biochemical parameters of sepsis, as well as rectal temperature confirmed the state of sepsis (Table 3).

Table 2

Average clinical rat sepsis score for the whole group of animals (n = 12)

Animals with sepsis (%)	Score
22	3 (mild sepsis)
78	4–6 (severe sepsis)

Table 3

Average rectal temperature and values of biochemical indicators of sepsis for the whole group of animals before the induction of sepsis and at the moment of sacrificing (n = 12)

Parameter	Before sepsis mean ± SD	At the moment of sacrificing mean ± SD
Rectal temperature (°C)	36.9 ± 0.4	38.6 ± 0.8
CRP (ng/mL)	325.74 ± 23.46	527.86 ± 20.67
PCT (pg/mL)	76.35 ± 10.84	200.54 ± 38.21

CRP – C-reactive protein; PCT – procalcitonin.

Cardiac function ex vivo

Cardiodynamic parameters for the assessment of cardiac function in *ex vivo* model are presented in Figure 1. As it can be seen, there was no significant difference in dp/dt max during all CPPs among the groups. Dp/dt min was higher in the control group (CPPs = 60 – 100 cm H_2O) than in the Sepsis and Sepsis + HBO group but without statistical significance. The SLVP and HR values were almost similar in all groups, while the DLVP was insignificantly higher in the control group. On the other hand, CF was statistically significantly higher ($p < 0.05$) in the Sepsis group than in the Sepsis + HBO group and also in the control group in comparison to other two groups at almost all CPPs (60–120 cm H_2O).

Cardiac oxidative stress

Biomarkers from coronary venous effluent as indicators of cardiac oxidative stress are presented in Figure 2. The values of O_2^- were significantly lower in the Sepsis + HBO group compared to the Sepsis group (at CPPs = 40, 60 and 80 cm H_2O, $p < 0.05$), while between the control and the Sepsis + HBO group there were no statistical differences. Similarly, the H_2O_2 values were lower in the Sepsis + HBO group compared to the Sepsis group with strong

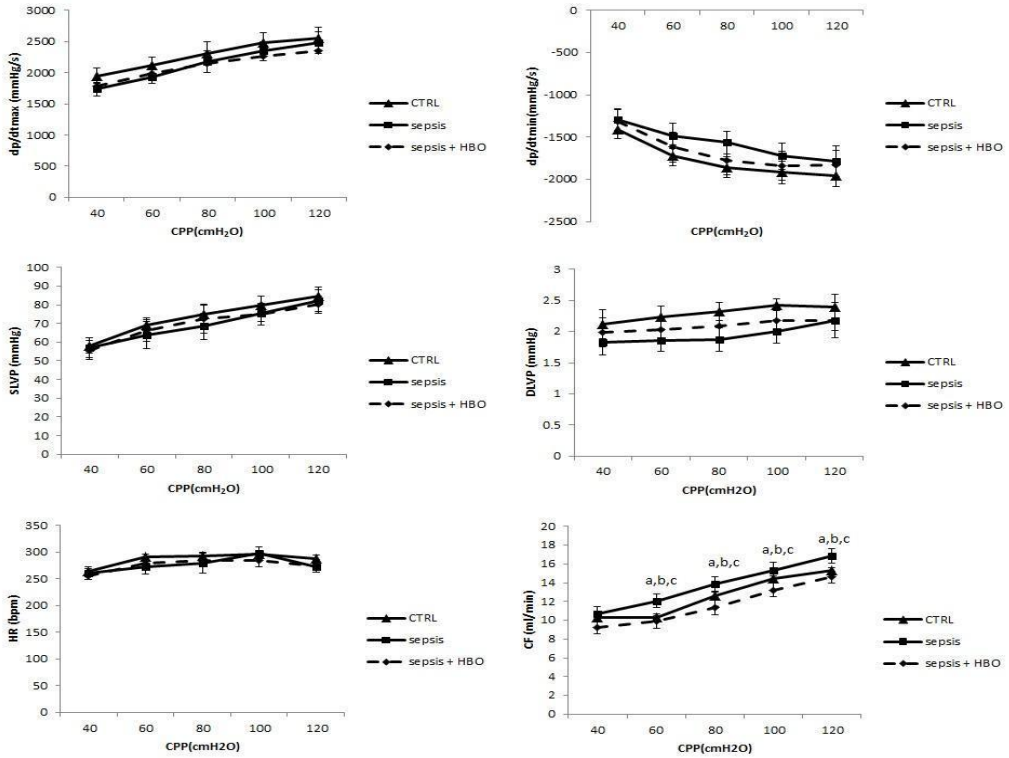

Fig. 1 – Parameters of cardiac function *ex vivo* (data are presented as mean values ± standard deviation)

dp/dtmax – maximum rate of pressure development; dp/dtmin – minimum rate of pressure development; SLVP – systolic left ventricular pressure; DLVP – diastolic left ventricular pressure; HR – heart rate; CF – coronary flow; CPP – coronary perfusion pressure.

Statistically significant differences ($p < 0.05$) among the groups at the same coronary perfusion pressure (CPP) are marked as follows: a – comparison between the control group (CTRL) and the group of rats with sepsis (the Sepsis group); b – comparison between the control group (CTRL) and the group of rats with sepsis treated with HBO (the Sepsis + HBO group); c – comparison between the group of rats with sepsis (the Sepsis group) and the group of rats with sepsis treated with HBO (the Sepsis + HBO group).

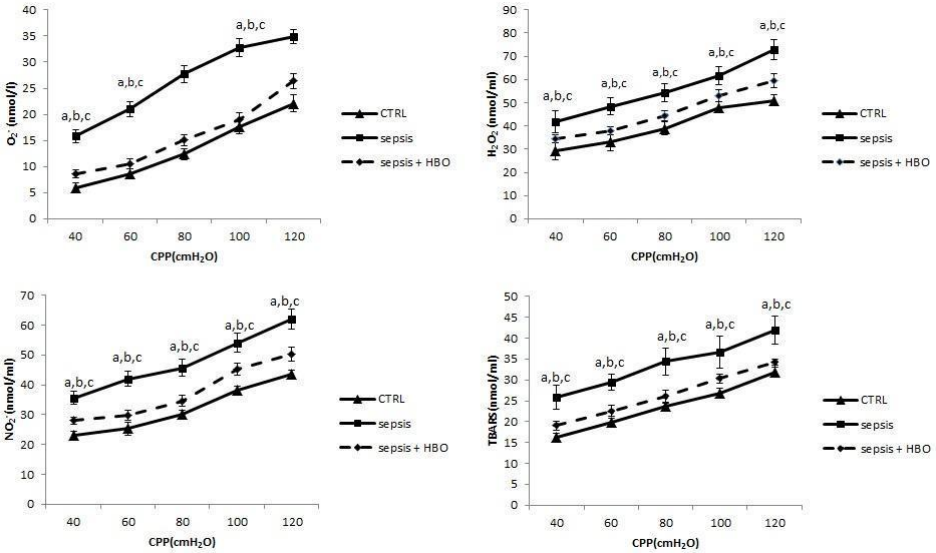

Fig. 2 – Parameters of cardiac oxidative stress (data are presented as mean values ± standard deviation).

O_2^- – superoxide anion radical; H_2O_2 – hydrogen peroxide; NO_2^- – nitrites; TBARS – thiobarbituric acid reactive substances (index of lipid peroxidation).

Statistically significant differences ($p < 0.05$) between the groups at the same coronary perfusion pressure (CPP) are marked as follows: a – comparison between the control group (CTRL) and the group of rats with sepsis (the Sepsis group); b – comparison between the control group (CTRL) and the group of rats with sepsis treated with HBO (the Sepsis + HBO group); c – comparison between the group of rats with sepsis (the Sepsis group) and the group of rats with sepsis treated with HBO (the Sepsis + HBO group.

statistical significance at all CPPs ($p < 0.01$), without significant differences between the control and the Sepsis + HBO group. The same trend in the Sepsis + HBO group had values of NO_2^- with statistical significance at all CPPs ($p < 0.05$). As in previous cases, TBARS values were also significantly lower in the Sepsis + HBO group (CPPs = 40–100 cm H_2O ($p < 0.05$)) compared to the Sepsis group without significant differences between the control and the Sepsis + HBO group.

Systemic oxidative stress

The values of pro-oxidants from plasma of both groups of rats are presented in Figure 3. Unlike cardiac oxidative stress markers, in the values of all measured systemic pro-oxidants, a significant difference between the Sepsis group and the Sepsis + HBO group was not observed. Namely, the values of NO_2^- and H_2O_2 were insignificantly lower in the Sepsis group, while the values of O_2^- and TBARS were insignificantly lower in the Sepsis + HBO group. Compared to the control group, the values of pro-oxidant were significantly higher in both, the Sepsis and Sepsis + HBO groups. In addition, the values of antioxidant enzymes from erythrocyte lysate are presented in Figure 4. Although it can be seen that SOD and GSH values were higher in the Sepsis + HBO group, there was no statistical confirmation. The CAT values were also without significant difference between the Sepsis and the Sepsis + HBO group, but compared to the control group, the values of this antioxidant enzyme were significantly lower in the Sepsis + HBO group.

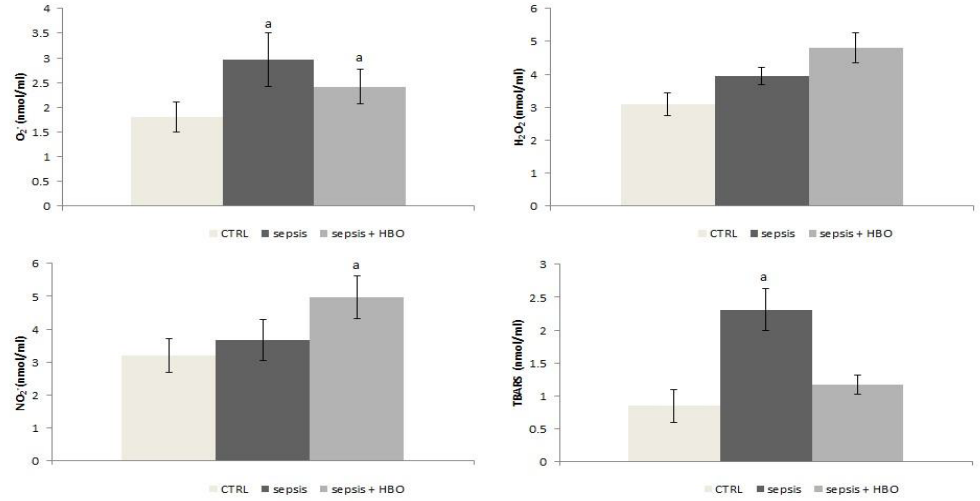

Fig. 3 – The values of pro-oxidants in plasma samples (data are presented as mean values ± standard deviation).
O_2^- – superoxide anion radical; H_2O_2 – hydrogen peroxide; NO_2^- – nitrites; TBARS – thiobarbituric acid reactive substances (index of lipid peroxidation).
Statistically significant differences ($p < 0.05$) are marked as follows:
a – statistical significance difference compared to the control group (CTRL).

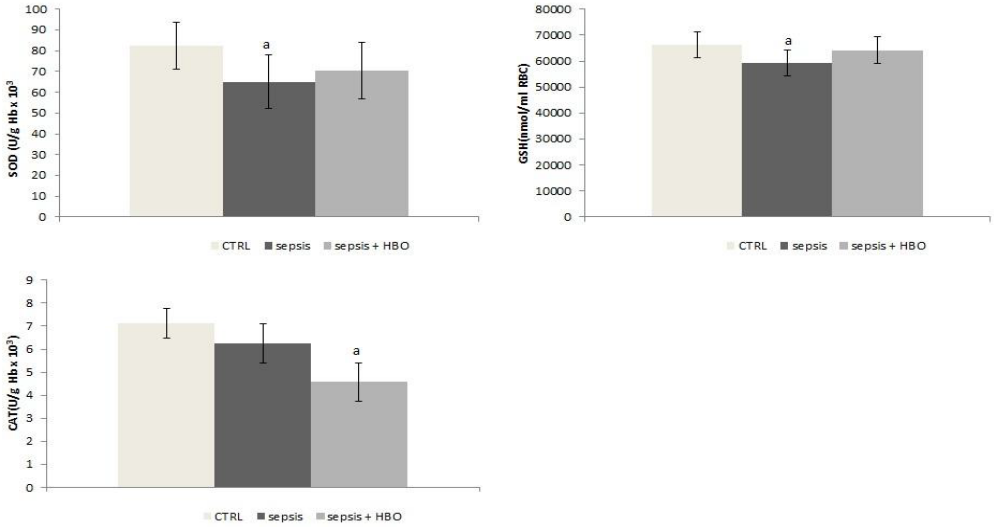

Fig. 4 – The values of antioxidant enzymes in erythrocyte lysate samples (data are presented as mean values ± standard deviation).
SOD – superoxide dismutase; CAT – catalase; GSH – glutathione.
Statistically significant differences ($p < 0.05$) are marked as follows:
a – statistical significance difference compared to the control group (CTRL).

Discussion

The present study aimed to assess the effects of HBO on cardiac function as well as cardiac and systemic oxidative state in rats with sepsis. The estimation of pro-oxidative and antioxidative markers was chosen to estimate a potential role of oxidative stress in the changes of cardiac function during sepsis and after HBO pretreatment. The function of the heart from aspect of cardiodynamics in sepsis and a possible impact of HBO were almost uninvestigated, with poor and heterogeneous data in the literature.

Cardiac dysfunction is a consequence of severe sepsis and is characterized by impaired contractility, diastolic dysfunction, as well as reduced cardiac index and ejection fraction (EF) [26]. The mechanisms involved in adverse effects of sepsis on myocardial function are little-known. Inflammatory mediators through impaired calcium turnover can lead to the alteration of cardiomyocyte contraction [26]. Other factors imply diminished β-adrenergic stimulation or reduced ATP production, which both cause cardiomyocyte dysfunction [27, 28].

On the other hand, HBO was introduced in recent years as a promising tool in the treatment of sepsis. The beneficial influence of HBO on sepsis is based on the enhancement of killing capacity of leukocytes, which substantially depends on the amount of oxygen [29]. Although some studies highlighted the positive effects of HBO pretreatment on kidney and liver injury in a model of rat sepsis [30], there are no data regarding the heart tissue.

In this study we evaluate the function of the isolated rat heart through the whole set of different cardiac parameters. In that manner, our results showed that dp/dt max, as an indirect indicator of the inotropic properties of the heart, was changed neither after sepsis nor after the HBO therapy. Similarly, dp/dt min, as an indirect indicator of lusitropic properties of the heart, was also unchanged in sepsis with a slight improvement after HBO, but still not enough for statistical confirmation. The same results were noticed with all other parameters, except CF. Namely, coronary endothelial response in healthy animals and after HBO treatment was weaker (Figure 1). However, coronary perfusion was in all groups in physiological range for this kind of protocol. These findings pointed out that hyperbaric oxygenation limited the effect on coronary endothelium and not on cardiomyocytes. In addition, it can be assumed that the longer time of exposure or different HBO protocols may achieve other effects.

The possible explanation for these results could be the fact that septic animals often develop tachycardia that progressed, especially in non-survivors. This includes mechanisms such as sympathetic overstimulation, despite the use of continuous opioid analgesia to manage pain [31]. It was previously reported that stroke volume and heart rate could be good predictors of the early phase of sepsis in a 3-day rat model of fecal peritonitis, which is the same design we used [31].

In the other part of the study we evaluated the possible impact of HBO on cardiac and systemic redox state during sepsis and estimated the role of oxidative stress in achieved effects. As expected, cardiac oxidative markers were the lowest in healthy animals indicating correlation between sepsis and increased oxidative stress within the heart. In terms of septic conditions, our findings showed the evident and strong depressed release of all investigated pro-oxidants in isolated rat hearts (Figure 2) after HBO treatment, indicating powerful protective influence of this procedure. When considering these results in light of rat cardiodynamics, it can be assumed that beneficial effects of HBO are firstly seen on molecular levels and that for functional improvement, longer time of HBO exposure is needed. The mechanisms of positive HBO impact on the cardiac oxidative status are not easy to explain. It seems that in the heart, exogenously derived hyperbaric oxygen is some which succeeded in suppressing the production of endogenous reactive oxygen species, but this claim is difficult to prove.

Unlike this, we did not find any changes in systemic oxidative status (Figures 3 and 4). However, although insignificant, noticed trend of increased activity of SOD and GSH level as well as a drop in the production of O_2^- and TBARS, pointed out that perhaps for more prominent results longer duration of exposure to HBO and/or higher ATA could be applied.

Most of the literature data covering the impact of HBO on oxidative damages during sepsis are gathered from animal models focused on liver and kidney. It is previously documented that hyperbaric oxygen applied at 2 ATA for 60 min and 1, 4, 9, and 24 h after the induction of sepsis, reduces synthesis of free radicals and mortality in rats [32]. The difference in comparison with results of our study could be the fact that authors used a lipopolysaccharide model of sepsis and a different protocol of HBO therapy. Moreover, we followed the animals for 72 hours after the induction of sepsis with intense and frequent HBO sessions.

Others investigated the effects of HBO on rat renal damage and oxidative stress markers after the induction of sepsis with an intraperitoneal injection of *Escherichia coli* cells (2.1×10^9) while HBO treatment was conducted through five sessions of 2 ATA at intervals of 6 h [33]. It was found that hyperbaric oxygen increased SOD and CAT activity and consequently reduced oxidative damages of the kidney induced by sepsis [33].

Oter et al. [34] assessed the effects of HBO on liver function and morphology as well as oxidative status in rats with sepsis caused by intraperitoneal application of *Escherichia coli* cells (2.1×10^9). In that study, HBO (which applied as six sessions at 2 ATA for 90 min at 6h intervals) in combination with cefepime reverses sepsis-induced both histopathological and functional changes of liver potentially through improved antioxidant activity.

Interestingly, the newest researches support the approach that for the best antioxidative results in sepsis, HBO should be used along with antibiotic therapy [35]. All of these studies partially correlate with the present investigation along with the fence that studies differ in sepsis model and HBO protocols.

Conclusion

To the best of our knowledge, this is one of only few studies that estimate the influence of hyperbaric oxygenation on cardiac function and coronary circulation during septic conditions. In that sense, the findings of the present research may be an important basis for designing future experiments, as well as clinical investigations. In the present study, we showed that HBO treatment was not associated with improved cardiac function and coronary perfusion, while it expressed promising beneficial effects on cardiac oxidative stress. A deeper assessment of this topic including underlying molecular mechanisms requires further investigations.

Acknowledgment

This work was supported by the Junior project No 03/19 of the Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia.

REFERENCES

1. *Gyawali B, Ramakrishna K, Dhamoon AS*. Sepsis: The evolution in definition, pathophysiology, and management. SAGE Open Med 2019; 7: 2050312119835043.
2. *Manfredini A, Constantino L, Pinto MC, Michels M, Burger H, Kist LW*, et al. Mitochondrial dysfunction is associated with long-term cognitive impairment in an animal sepsis model. Clin Sci (Lond) 2019; 133(18): 1993–2004.
3. *Rello J, Valenzuela-Sánchez F, Ruiz-Rodriguez M, Moyano S*. Sepsis: A review of advances in management. Adv Ther 2017; 34(11): 2393–411.
4. *Fattahi F, Ward PA*. Complement and sepsis-induced heart dysfunction. Mol Immunol 2017; 84: 57–64.
5. *Gupta A, Brahmbhatt S, Kapoor R, Loken L, Sharma AC*. Chronic peritoneal sepsis: myocardial dysfunction, endothelin and signaling mechanisms. Front Biosci 2005; 10: 3183–205.
6. *Chopra M, Sharma AC*. Distinct cardiodynamic and molecular characteristics during early and late stages of sepsis-induced myocardial dysfunction. Life Sci 2007; 81(4): 306–16.
7. *Chopra M, Sharma AC*. Apoptotic cardiomyocyte hypertrophy during sepsis and septic shock results from prolonged exposure to endothelin precursor. Front Biosci 2007; 12: 3052–60.
8. *Babul S, Rhodes EC*. The role of hyperbaric oxygen therapy in sports medicine. Sports Med 2000; 30(6): 395–403.
9. *Poff AM, Kernagis D, D'Agostino DP*. Hyperbaric environment: oxygen and cellular damage versus protection. Compr Physiol 2016; 7(1): 213–34.
10. *Bennett M, Best TM, Babul S, Taunton J, Lepawsky M*. Hyperbaric oxygen therapy for delayed onset muscle soreness and closed soft tissue injury. Cochrane Database Syst Rev 2005; (4): CD004713.
11. *Demchenko IT, Zhilyaev SY, Moskvin AN, Krivchenko AI, Piantadosi CA, Allen BW*. Baroreflex-mediated cardiovascular responses to hyperbaric oxygen. J Appl Physiol 2013; 115(6): 819–28.
12. *Bergo GW, Tyssebotn I*. Cardiovascular effects of hyperbaric oxygen with and without addition of carbon dioxide. Eur J Appl Physiol Occup Physiol 1999; 80(4): 264–75.
13. *Halbach JL, Prieto JM, Wang AW*. Early hyperbaric oxygen therapy improves survival in a model of severe sepsis. Am J Physiol Regul Integr Comp Physiol 2019; 317(1): R160–8.
14. *Tai PA, Chang CK, Niu KC, Lin MT, Chiu WT, Lin JW*. Reduction of ischemic and oxidative damage to the hypothalamus by hyperbaric oxygen in heatstroke mice. J Biomed Biotechnol 2010; 2010: 609526.
15. *Zolfaghari PS, Pinto BB, Dyson A, Singer M*. The metabolic phenotype of rodent sepsis: cause for concern? Intensive Care Med Exp 2013; 1(1): 25.
16. *Ozturk A, Yamanel L, Ozenc S, Ince M, Simsek K, Comert B,* et al. Comparison of the effects of hyperbaric oxygen and normobaric oxygen on sepsis in rats. Arch Clin Exp Surg 2016; 5(1): 7–12.
17. *Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR*. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal Biochem 1982; 126(1): 131–8.
18. *Pick E, Keisari Y*. A simple colorimetric method for the measurement of hydrogen peroxide produced by cells in culture. J Immunol Methods 1980; 38(1–2): 161–70.
19. *Ohkawa H, Ohishi N, Yagi K*. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95(2): 351–8.
20. *Auclair C, Voisin E*. Nitroblue tetrazolium reduction. In: *Greenwald RA*, editor. Handbook of methods for oxygen radical research. Boca Raton: CRC Press; 1985. p. 123–32.
21. *McCord JM, Fridovich I*. The utility of superoxide dismutase in studying free radical reactions. I. Radicals generated by the interaction of sulfite, dimethyl sulfoxide, and oxygen. J Biol Chem 1969; 244(22): 6056–63.
22. *Misra HP, Fridovich I*. The role of superoxide-anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972; 247(10): 3170–5.
23. *Beutler E*. Catalase. In: *Beutler E* editor. Red cell metabolism, a manual of biochemical methods. New York, NY, USA: Grune and Stratton; 1982. p. 105–6.
24. *Beutler E*. Reduced glutathione (GSH). In: *Beutler E* editor. Red cell metabolism, a manual of biochemical methods. New York, NY, USA: Grune and Stratton; 1975. p. 112–4.
25. *Zhai X, Yang Z, Zheng G, Yu T, Wang P, Liu X,* et al. Lactate as a potential biomarker of sepsis in a rat cecal ligation and puncture model. Mediators Inflamm 2018; 2018: 8352727.
26. *Drosatos K, Lymperopoulos A, Kennel PJ, Pollak N, Schulze PC, Goldberg IJ*. Pathophysiology of sepsis-related cardiac dysfunction: driven by inflammation, energy mismanagement, or both? Curr Heart Fail Rep 2015; 12(2): 130–40.
27. *de Montmollin E, Aboab J, Mansart A, Annane D*. Bench-to-bedside review: beta-adrenergic modulation in sepsis. Crit Care 2009; 13(5): 230.
28. *Drosatos K, Khan RS, Trent CM, Jiang H, Son NH, Blaner WS*, et al. Peroxisome proliferator-activated receptor-γ activation prevents sepsis-related cardiac dysfunction and mortality in mice. Circ Heart Fail 2013; 6(3): 550–62.
29. *Yang ZJ, Bosco G, Montante A, Ou XI, Camporesi EM*. Hyperbaric O_2 reduces intestinal ischemia-reperfusion-induced TNF-alpha production and lung neutrophil sequestration. Eur J Appl Physiol 2001; 85(1–2): 96–103.
30. *Chang KY, Tsai PS, Huang TY, Wang TY, Yang S, Huang CJ*. HO-1 mediates the effects of HBO pretreatment against sepsis. J Surg Res 2006; 136(1): 143–53.
31. *Rudiger A, Dyson A, Felsmann K, Carré JE, Taylor V, Hughes S,* et al. Early functional and transcriptomic changes in the myocardium predict outcome in a long-term rat model of sepsis. Clin Sci (Lond) 2013; 124(6): 391–401.
32. *Lin HC, Wan FJ, Wu CC, Tung CS, Wu TH*. Hyperbaric oxygen protects against lipopolysaccharide-stimulated oxidative

stress and mortality in rats. Eur J Pharmacol 2005; 508(1–3): 249–54.
33. *Edremitlioğlu M, Kiliç D, Oter S, Kisa U, Korkmaz A, Coşkun O,* et al. The effect of hyperbaric oxygen treatment on the renal functions in septic rats: relation to oxidative damage. Surg Today 2005; 35(8): 653–61.
34. *Oter S, Edremitlioglu M, Korkmaz A, Coskun O, Kilic D, Kisa U,* et al. Effects of hyperbaric oxygen treatment on liver functions, oxidative status and histology in septic rats. Intensive Care Med 2005; 31(9): 1262–8.
35. *Bektas A, Ulusoy M, Mas MR.* Do late phase hyperbaric and normobaric oxygen therapies have effect on liver damage? An experimental sepsis model. Gen Med (Los Angel) 2019; 7(1): 324.

Received on October 26, 2019
Revised on December 9, 2019
Accepted December 10, 2019
Online First December, 2019