ORIGINAL SCIENTIFIC ARTICLE

SYSTEMIC REDOX STATUS OF RATS TREATED WITH DIFFERENT DOSES OF PERFLUOROCARBON BASED BLOOD SUBSTITUTE- PERFTORAN®

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ABSTRACT

The aim of this research was to examine the influence of the intraperitoneal application of PFT in different doses and regimen on systemic oxidative stress and activity of antioxidative enzymes in animals. Depending on whether the animals received only saline or PFT in different doses (8, 12, 16 ml/kg body weight), and time (1, 10, or 20 hours before sacrificing and blood sampling), all animals were divided into control or experimental groups. From plasma samples we measured following biomarkers of oxidative stress: superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), nitrites (NO_2^-), index of lipid peroxidation measured as TBARS (thiobarbituric acid reactive substances), and from hemolysate samples activity of the next enzymes: catalase (CAT), superoxide-dismutase (SOD) and reduced glutathione (GSH). All mentioned biochemical parameters of oxidative stress were determined spectrophotometrically (Shimadzu UV-1800UV-VIS spectrophotometer, Japan). Superoxide anion radical was a molecule very affected with the PFT administration. we observed the significantly higher activity of superoxide dismutase in all PFT treated groups in comparison with the CTRL group. The highest activity was observed in group treated with the 8 and 12 ml/kg of PFT nearly to sampling (1 hour). Catalase activity was significantly higher in PFT group in comparison with the CTRL, especially in PFT 16ml/kg group (1 hour). In comparison with the CTRL group, the total content of GSH was significantly lower in the groups treated PFT in dose of 16 ml/kg 1 hour and 10 hours before blood sampling. All these changes in oxidative stress markers seems to be very clear, but we can observe that almost all changes are induced in 1 hour after PFT administration. Probably, PFT solution has short-term protective effects on reducing oxidative stress, but no long term-effects. Maybe the chemical and biological instability of PFT solution could be a reason for that transient antioxidative effects, and developing the nano-formulation of PFT could be potential option for resolving the problem with poor pharmacodynamic of PFT.

Keywords:*Perfluorocarbon, oxidative stress, antioxidative enzymes, blood samples, rat.*

INTRODUCTION

The development of pefluorocarbon solution (PFT) began during 80' years, when it is suggested that PFT as infusion could be blood substitute and transport oxygen in vivo. As a chemically and biologically inert, pure PFT is not suitable for injection into vascular system, but the nano-emulsion as a best formulation could be used because of the faster clearance, longer retention time in organs and lower risk for side effects (1, 2)

In history, the first experiment with PFT was done by Clark and Gollan who kept Langendorff-heart beating by perfusing it with pure PFT (3). After that, in 1967, Sloviter suggested that component of PFE must be prepared as emulsion, because of the insolubility of glucose and salts, so he was the first scientist who emulsify PFE in water (4, 5). That was a challenge because of the many different components in this solution, such as lipids, glucose, salts, proteins and water.

The second challenge was stability of PFE emulsion. We know that emulsions are metastable because reaches thermodynamic equilibrium only after complete phase separation. Nano emulsion of PFT shows droplets diameter below 1 μm, and it is more stable than micro emulsions.

Almost 30 years ago, PFT emulsion was developed named as Fluosol-DA® which was the first generation of these syntetic solutions. The first use of this commercially-available product was reported in Japan with infusion of 500 ml and 1000 ml given to patients with severe gastrointestinal bleeding and surgery-related blood loss for esophageal cancer, respectively. Fluosol-DA® was only FDA approved PFT for clinical use (6, 7).

After that, in future years were sintethyzed another PFT solutions but not FDA-approved. Most of them were reached only phase II trials. Some of them were Oxygent®, Oxycyte® and they are still in preclinical testing.

In 1996 in Russia, Professor S.I. Vorobyev and her collaborators registered perfluorocarbon emulsion Perftoran® for wide clinical use for the first time in the world (8, 9).

Definitely, PFT solutions are products that in theory seem to be a viable option for clinical use. However, due to the short stability of approximately 8 hours it is difficult to use it in coronary balloon angioplasty, the indication for which the product has gained its approval, and that led to FDA removing Fluosol® from the market in 1994. Developing of the new nano emulsion seems to PFT, especially Peftoran-plus (Ftoremulsion III, Russia) could be approved for clinical use in the future.

On the other hand, examining the association of PFT and level of oxidative stress is still actual, since it is clear that PFT emulsions deliver oxygen to the tissues as a hemoglobin-based carrier.

Oxidative stress as an imbalance of free radicals and antioxidants in organism is also something that could be associated with the effects of PFT. Oxidative stress (OS) has the ability to damage different molecules and cellular structures, altering the correct function of organs and systems (10-13). OS accumulates in the body by endogenous and exogenous mechanisms. Increasing evidence points to the involvement of OS in the physiopathology of various chronic diseases that require prolonged periods of pharmacological treatment (10-13).

In previous studies, PFT was mentioned as potential preventive strategy for ischemia/reperfusion injuries for example in lung graft preservation (14).

Also, it is suggested that PFT could decrease production of inflammatory markers and stress oxidative markers, decrease lung oedema and hydroxyproline level, as well as attenuate histopathological changes and fibrosis (15).

Based on previous literature data, the greatest future of PFT may be not in the treatment of anemia, but in improving the microcirculatory oxygen delivery for treatment of ischemic tissues. For example, PFT solution could be a choice of treatment in stroke, myocardial infarction, ischemic extremities etc.

According to that, the aim of this research was to examine the influence of the intraperitoneal application of PFT in different doses and regimen on systemic oxidative stress and activity of antioxidative enzymes in animals.

MATERIAL AND METHODS

Ethics approval

All research procedures were carried out in strict accordance with the European Union Directive for the welfare of laboratory animals (No. 2010/63/EU) and approved by the Ethics Committee of Faculty of Medical Sciences, University of Kragujevac, Serbia.

Study design

In this study we used male Wistar albino rats (10 weeks old, weighed 200 ± 20 g on average) who were housed in a temperature-controlled vivarium (22 ± 2 ° C), with 12-hour alternating light-dark cycles. Standard food and water were available to the animals ad libitum.

This study was aimed to determine the dose of 20% perfluoroemulsion (PFT) on redox parameters in blood samples of rats. Depending on whether the animals received only saline or PFT in different doses (8, 12, 16 ml/kg body weight), and time (1, 10, or 20 hours before sacrificing and blood sampling), all animals were divided into control or experimental groups:

Control groups:

1. Control (CTRL) group - without application of PFT solution (n=6)

Experimental groups:

1. Group PFT 8 ml/kg - 1 hour before sampling (n=6)
2. Group PFT 12 ml/kg - 1 hour before sampling (n=6)
3. Group PFT 16 ml/kg - 1 hour before sampling (n=6)
4. Group PFT 16 ml/kg - 10 hours before sampling (n=6)
5. Group PFT 16 ml/kg - 20 hours before sampling (n=6).

Perfluorocarbon-based blood substitute (PFT)

For the purposes of the study, 20% perfluorocarbon emulsion (Perftoran®) was used, which was prepared ex tempore by dissolving equal volumes of 20% PFE for intravenous administration. The chemical composition of the Perfluorocarbon-based blood substitute is: 13,0 g (6,5 ml) of Perfluorodecalin, 6,5 g (3,25 ml) of Perfluoro-N-4- (methylcyclohexyl) - piperidine, 4,0 g of Proxanol-268, 0,6 g of Sodium chloride (NaCl), 0.039 g of Potassium chloride (KCL), 0.019 g of Magnesium chloride ($MgCl_2$), 0.065 g of Sodium bicarbonate ($NaHCO_3$), 0.02 g of Sodium phosphate monobasic (NaH_2PO_4), 0,2 g of glucose and 100 ml Distilled Water (H_2O). This solution Peftoran® was purchased in the Research Laboratory of Biological and Physico-Chemical Study of perfluorocarbons, Russian Federation.

Preparation of blood samples

Rats were anesthetized with a combination of ketamine and xylazine and sacrificed by decapitation using a guillotine for small laboratory animals. Immediately after sacrifice, a whole blood sample was collected in tubes with 3.8% sodium citrate. The blood sample was gently agitated to thoroughly mix the sodium citrate with the blood. Subsequently, the sample was centrifuged three times at 3000 rpm for 10 min at room temperature and dissolved with distilled water for preparing the hemolysate.

Spectrofotometric determination of biomarkers of oxidative stress

From plasma samples we measured following biomarkers of oxidative stress: superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), nitrites (NO_2^-), index of lipid peroxidation measured as TBARS (thiobarbituric acid reactive substances), and from hemolysate samples activity of the next enzymes: catalase (CAT), superoxide-dismutase (SOD) and reduced glutathione (GSH). All mentioned biochemical parameters of oxidative stress were determined spectrophotometrically (Shimadzu UV-1800UV-VIS spectrophotometer, Japan).

O_2^- concentrations were determined according to Auclair, by using the NTB (Nitro Blue Tetrazolium) reagent in TRIS buffer (assay mixture) with the sample, while the measurement was performed at a wavelength of 530 nm (16).

The determination of H_2O_2 was based on the oxidation of phenol red by H_2O_2 which is catalysed by horseradish peroxidase. The level of H_2O_2 was then measured at 610 nm of wavelength (17).

Nitrites level was measured in order to indirectly asses nitric oxide level. Nitrites were quantified by method according to Green using the Griess-reagent (18). The sample was precipitated with 30% sulfo-salicylic acid, vortexed for 30 min, and centrifuged at 30009g. Equal volumes of the supernatant and Griess's reagent, containing 1% sulfanil-amide in 5% phosphoric acid/0.1% napthalene ethylenediamine-dihydrochloride, were added and incubated for 10 min in the dark and measured at 543 nmol/l.

Index of lipid peroxidation in the blood samples was estimated via measuring of TBARS using 1% thiobarbituric acid (TBA) in 0.05M sodium hydroxide (NaOH) incubated with the sample at 100 C for 15 min and then measured at 530 nm (19).

Determination of antioxidative enzymes (superoxide dismutase, catalase, reduced glutathione) in the lysate samples from women with PCOS

SOD activity was evaluated by using epinephrine method according to Beutler (20). Heart tissue homogenate sample was first mixed with carbonate buffer, and afterwards epinephrine was added to the mixture. SOD activity was measured at 470 nm of wave length and was expressed as U/ml/Hb of hemolysate.

Determination of antioxidant enzyme CAT was carried out according to Aebi`s method (21). CAT buffer, prepared lysate sample, and 10 mM H_2O_2 were used for CAT determination. The activity of CAT was measured spectrophotometrically at 360 nm of wave length and was expressed in U/ml/Hb of hemolysate.

The level of GSH was determined according to Beutler (22). The method involves the reaction of GSH oxidation with 5.5-dithio-bis-6.2-nitrobenzoic acid. The level of GSH was measured spectrophotometrically at 420 nm of wave length and was expressed in U/ml/Hb of hemolysate.

Statistical analysis

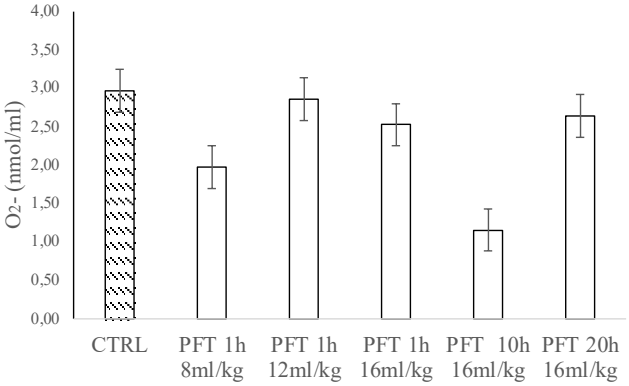
All data were presented in form of tables and figures. Results are presented and analyzed by descriptive analyses (means, standard deviations and standard errors of mean). Analytical statistical analysis is performed using Kruskal-Wallis and Tukey post hoc test for comparison the changes in percent between groups. Statistical significance was set at the level of 0.05. Statistical analysis was done using IBM SPSS statistical software version 26.0.

RESULTS

The effects of PFT on superoxide anion radical, hydrogen peroxide, nitric oxide and index of lipid peroxidation

From the figure 1, we can see the mean levels of concentrations of superoxide anion radical. Treatment with PFT in dose of 16ml/kg and 8 ml/kg induced the most significant lower levels of this marker in comparison with untreated rats (CTRL group) (Fig. 1; Table 1).

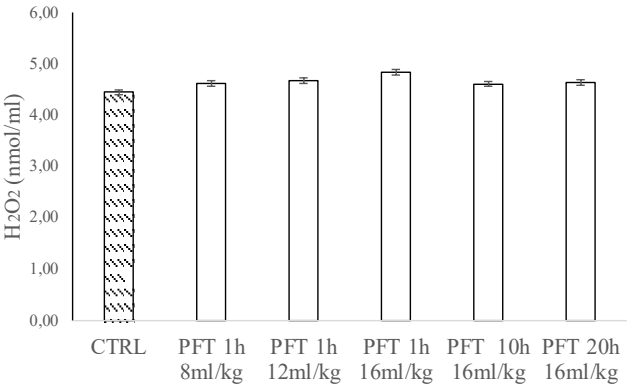
Figure 1. The mean values of superoxide anion radical from plasma samples in different groups.



Results are presented as mean ± standard error of mean.

On the other hand, the concentration of plasma hydrogen peroxide was not statistically significant different between groups (Fig. 2; Table 1).

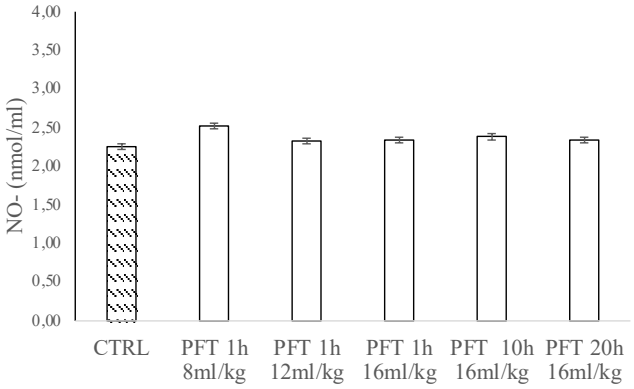
Figure 2. The mean values of hydrogen peroxide from plasma samples in different groups.



Results are presented as mean ± standard error of mean.

Bioavailability of nitric oxide was statistically significant higher in group treated with 8ml/kg of PFT 1 hour before sacrificing in comparison with the control group. In other groups, NO was not statistically significantly altered (Fig. 3; Table 1).

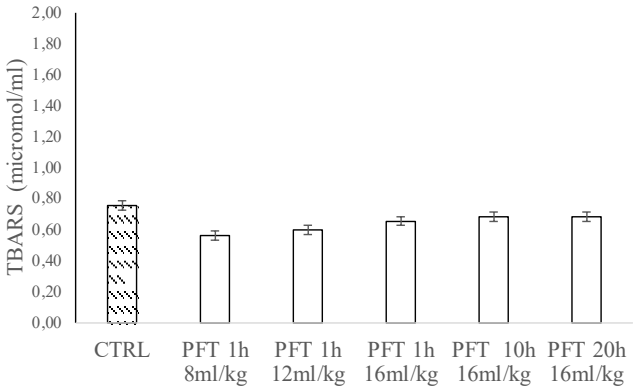
Figure 3. The mean values of nitric oxide from plasma samples in different groups.



Results are presented as mean ± standard error of mean.

From the Figures 4 and Table 1, we can see the lower levels of index of lipid peroxidation in group treated with 8 ml/kg and 12 ml/kg of PFT given 1 hour before sampling. Other values were similar with the values in CTRL group (Fig. 4; Table 1).

Figure 4. The mean values of index of lipid peroxidation measured as TBARS from plasma samples in different groups.

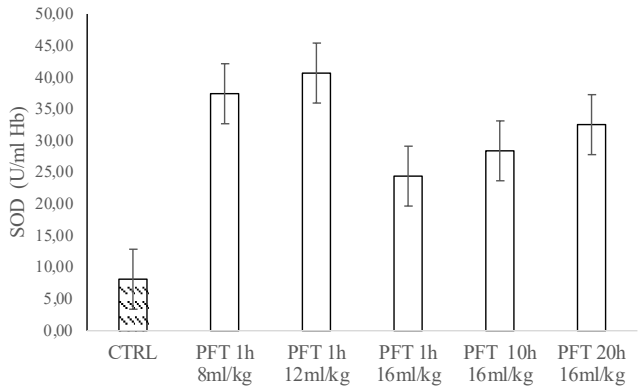


Results are presented as mean ± standard error of mean.

The effects of PFT on superoxide superoxide dismutase, catalase and reduced glutathione

From the Figure 5 and Table 1, we observed the significantly higher activity of superoxide dismutase in all PFT treated groups in comparison with the CTRL group. The highest activity was observed in group treated with the 8 and 12 ml/kg of PFT nearly to sampling (1 hour) (Fig. 5; Table 1).

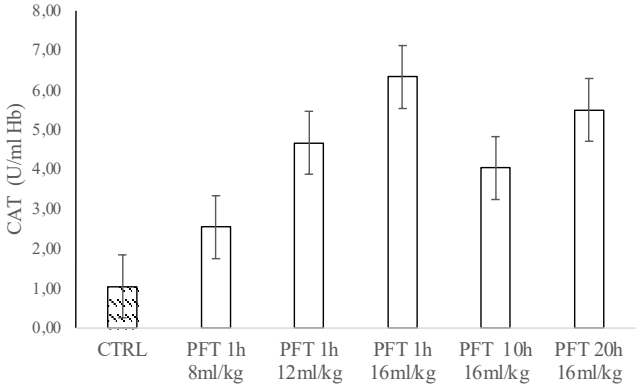
Figure 5. Activity of superoxide dismutase from hemolysate samples in different groups.



Results are presented as mean ± standard error of mean.

Also, catalase activity was significantly higher in PFT group in comparison with the CTRL, especially in PFT 16ml/kg group (1 hour) (Fig. 6).

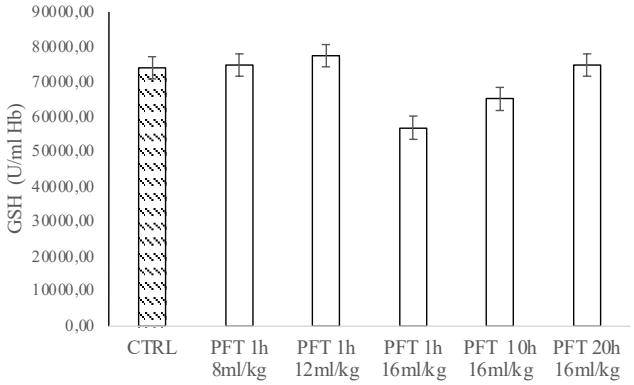
Figure 6. Activity of catalase from hemolysate samples in different groups.



Results are presented as mean ± standard error of mean.

In comparison with the CTRL group, the total content of GSH was significantly lower in the groups treated PFT in dose of 16 ml/kg 1 hour and 10 hours before blood sampling (Fig. 7).

Figure 7. The mean content of reduced glutathione from hemolysate samples in different groups.



Results are presented as mean ± standard error of mean.

Table 1. Comparison of the values of PFT-treated rats with the values in CTRL group.

Comparison	O2-	H2O2	NO-	TBARS	SOD	CAT	GSH
CTRL vs. PFT 1h 8ml/kg	p=0.02*	p>0.05	p=0.038*	p=0.041*	p<0.001**	p=0.002**	p>0.05
CTRL vs. PFT 1h 12ml/kg	p>0.05	p>0.05	p>0.05	p=0.046*	p<0.001**	p<0.001**	p>0.05
CTRL vs. PFT 1h 16ml/kg	p=0.03*	p>0.05	p>0.05	p>0.05	p<0.001**	p<0.001**	p=0.021*
CTRL vs. PFT 10h 16ml/kg	p=0.02*	p>0.05	p>0.05	p>0.05	p<0.001**	p<0.001**	p=0.028*
CTRL vs. PFT 20h 16ml/kg	p>0.05	p>0.05	p>0.05	p>0.05	p<0.001**	p<0.001**	p>0.05

Statistical analysis was done using Kruskal-Wallis Test.
Single asterisk (*) represents statistical significance, while double asterisk (**) represents high statistical significance.

DISCUSSION

The main aim of this research was to examine the influence of the intraperitoneal application of PFT in different doses and regimen on systemic oxidative stress and activity of antioxidative enzymes in animals. Actually, this study is a second part of our research of the effects of PFT on cardiovascular system, where we reported that PFT administered before ischemia (1 hour) has less positive effects on myocardial function in an isolated rat heart model compared to earlier administration (10 and 20 hours). Also, the effects of 20% PFE are more pronounced if there is a longer period of time from application to ischemia, i.e., immediate application of PFE before ischemia (1 hour) gave the weakest effects on the change of cardiodynamics of isolated rat heart. Therefore, the future of PFE use is in new indications and application methods, and PFE can also be referred to as anti-hypoxic and anti-ischemic blood substitute with mild membranotropic effects (22).

Based on these conclusions, we continued our research and using standard methods for determining the concentrations of biomarkers of oxidative stress in rat blood, we evaluated the effects of PFT administration on their changes. Definitely, we have founded that PFE administration induced decreasing the superoxide anion radical and increasing the activity of antioxidative enzymes, which confirms the antioxidative role of PFT in vivo in comparison with the control conditions (Figs. 1-7; Table 1).

Superoxide anion radical was a molecule very affected with the PFT administration. Production of superoxide anion radical is essential for the life of aerobic organisms who acts as a signaling molecule in many processes such as apoptosis, aging etc (24). All these processes are associated with the many serious diseases when we observe the lower scavenging of O_2-. Also, poor antioxidants capacity of organism is reason for the increased levels of free radicals, such as superoxide anion radical. In our study, PFT showed that has ability to decreased the level of this marker of oxidative stress.

Interestingly, nitric oxide was increased after PFT administration 1 hour before and in dose of 8ml/kg. As we know, NO is actually synthesized from arginine by the endothelial isoform of NO-synthase (eNOS) in response to an appropriate stimulus. Endothelial NO is dispersed in vascular smooth muscle cells where cytosolic guanylate cyclase is activated and increases the production of cyclic guanosine monophosphate, leading to smooth muscle cells relaxation. Loss of endothelium-mediated vasodilator capacity is considered one of the earliest manifestations of cardiovascular damage and precedes the formation of atherosclerotic plaques.

Previous study conducted by Bekyarova et al, concluded that the combined application of alpha-tocopherol and FC-43 perfluorocarbon emulsion immediately after thermal skin injury in rats increases plasma antioxidant capacity, decreases free radical mediated damage of erythrocytes and suppresses their aggregation on the third hour after the injury (25).

Also, Zhang et al reported about the antioxidant potential of PFT. Their research showed that PFC reduced A549 cell damage caused by blast injury. Also, suggested the potential mechanism which may be associated with the following signaling pathways:

the signaling pathways of NF-κB and MAPK, which inhibit inflammation and reactive oxygen species (ROS); and 2) the signaling pathways of Bcl-2/Bax and caspase-3, which inhibit apoptosis (26).

Also, in the pathophysiology of neurological disease oxidative stress has an important role. Previous authors confirmed the preventive role of PFT in schizophrenia (27).

Furthermore, in the state of redox imbalance, high oxidative stress could be reduced by antioxidative defense system, which is crucial to reduce risk factors for the development the disease. In our study, we observed the significantly higher activity of superoxide dismutase in all PFT treated groups in comparison with the CTRL group. The highest activity was observed in group treated with the 8 and 12 ml/kg of PFT nearly to sampling (1 hour) (Fig. 5; Table 1). Also, catalase activity was significantly higher in PFT group in comparison with the CTRL, especially in PFT 16ml/kg group (1 hour) (Fig. 6). In comparison with the CTRL group, the total content of GSH was significantly lower in the groups treated PFT in dose of 16 ml/kg 1 hour and 10 hours before blood sampling (Fig. 7).

In addition, both downregulation of components of the antioxidant synthesis and increases of reactive oxygen species (ROS) have been observed in patients with ischemic disease (28). So, administration the PFT in animals could decrease oxidative stress and improve antioxidant capacity, even for the short time (few hours). Other authors also confirmed these results, such as reducing the production of free radicals and the number of pulmonary structural where changes resulting from cold ischemia (28, 29).

PFT solution which we used, is consist of 20% of total perfluorocarbon in 100 ml of solution, and compared with the other available PFTs, it is with lower percent of PFT. Other PFT are mostly higher concentration based PFT, so maybe that is a reason for the transient antioxidative effect in our study (30, 31). Dani et al investigated the effect of tidal liquid ventilation (TLV) compared to conventional mechanical ventilation (CMV) on oxidative lung damage in the setting of acute respiratory distress syndrome (ARDS). They concluded that animals treated with TLV showed lower oxidative lung damage compared to animals treated with CMV (32).

CONCLUSION

All these changes in oxidative stress markers seems to be very clear, but we can observe that almost all changes are induced in 1 hour after PFT administration. Probably, PFT solution has short-term protective effects on reducing

oxidative stress, but no long term-effects. Maybe the chemical and biological instability of PFT solution could be a reason for that transient antioxidative effects, and developing the nano-formulation of PFT could be potential option for resolving the problem with poor pharmacodynamic of PFT.

ETHICS APPROVAL

All research procedures were carried out in strict accordance with the European Union Directive for the welfare of laboratory animals (No. 2010/63/EU) and approved by the Ethics Committee of Faculty of Medical Sciences, University of Kragujevac, Serbia.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

None.

LITERATURE

1. Jägers J, Wrobeln A, Ferenz KB. Perfluorocarbon-based oxygen carriers: from physics to physiology. Pflugers Arch. 2021;473(2):139-50.
2. Bucala R, Kawakami M, Cerami A. Cytotoxicity of a perfluorocarbon blood substitute to macrophages in vitro. Science. 1983;220:965–97.
3. Castro CI, Briceno JC. Perfluorocarbon-based oxygen carriers: review of products and trials. Artif Organs. 2010;34:622–34.
4. Gollan F, Clark L. Organ perfusion with fluorocarbon fluid. Physiologist. 1966;9:H292
5. Sloviter HA, Kamimoto T. Erythrocyte substitute for perfusion of brain. Nature. 1967;216:458–60
6. Honda K, Hoshino S, Shoji M, Usuba A, Motoki R, Tsuboi M, et al. Clinical use of a blood substitute. N Engl J Med. 1980;303(7):391-2.
7. Vorobyev SI, Ivanitsky GR. Perfluorocarbon emulsion Perftoran - the plasma substite with gastransporting function. // VI Inter. Sympos. on Blood Substit. 1996; Canada. Montreal.
8. Vorobyev SI, Ivanitsky GR. Perfluorocarbon emulsion, stabilised proxanol. Preprint. Pushchino. 1994.
9. Winslow RM. Current status of blood substitute research: towards a new paradigm. J Intern Med. 2003;253(5):508-17.
10. Kerins DM. Role of the perfluorocarbon Fluosol-DA in coronary angioplasty. Am J Med Sci. 1994;307(3):218-21.
11. Rajendran P, Nandakumar N, Rengarajan T, Palaniswami R, Gnanadhas NE, Lakshminarasaiahet U, et al. Antioxidants and human diseases. Clin Chim Acta. 2014;436:332-47.
12. Brand MD. The sites and topology of mitochondrial superoxide production. Exp Gerontol. 2010;45:466-72.
13. Fujii H, Nakai K, Fukagawa M. Role of oxidative stress and indoxyl sulfate in progression of cardiovascular disease in chronic kidney disease. Ther Apher Dial. 2011;15(2):125-28.
14. Ferrari RS, Thomaz LDGR, Simoneti LEL, Ulbrich JM, Andrade CF. Effect of vaporized perfluorocarbon on oxidative stress during the cold ischemia phase of lung graft preservation. J Bras Pneumol. 2019;45(4):e20170288.
15. Khalighi Z, Rahmani A, Cheraghi J, Ahmadi MR, Soleimannejad K, Asadollahi R, Asadollahi K. Perfluorocarbon attenuates inflammatory cytokines, oxidative stress and histopathologic changes in paraquat-induced acute lung injury in rats. Environ Toxicol Pharmacol. 2016;42:9-15.
16. Auclair C, Voisin E. Nitroblue tetrazolium reduction. In: Greenvvald RA, editor. Handbook of methods for oxygen radical research. CRC Press, Boca Raton; 1985, p. 123–32.
17. Pick E, Keisari Y. A simple colorimetric method for the measurement of hydrogen peroxide produced by cells in culture. J Immunol Meth. 1980;38:161–70.
18. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite and [15 N] nitrate in biological fluids. Anal Biochem. 1982;126:131–8
19. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351–8.
20. 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;244:6056–63.
21. Beutler E. Catalase, red cell metabolism. In: Beutler E, editor. Manual of biochemical methods. Grune and Stratton, New York; 1982; p. 105.
22. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963;61:882-8.
23. Jakovljevic V, Vorobyev S, Bolevich S, Morozova E, Bolevich S, Saltykov A, et al. Dose-dependent effects of perfluorocarbon-based blood substitute on cardiac function in myocardial ischemia-reperfusion injury. Mol Cell Biochem. 2022;10.1007/s11010-022-04479-0. doi:10.1007/s11010-022-04479-0
24. Chiste RC, Freitas M, Mercadante AZ, Fernandes E. Superoxide Anion Radical: Generation and Detection in Cellular and Non-Cellular Systems. Curr Med Chem. 2015;22(37):4234-56.
25. Bekyarova G, Yankova T, Galunska B. Increased antioxidant capacity, suppression of free radical damage and erythrocyte aggrerability after combined application of alpha-tocopherol and FC-43 perfluorocarbon emulsion in early postburn period in rats. Artif Cells Blood Substit Immobil Biotechnol. 1996;24(6):629-41.
26. Zhang Z, Liang Z, Li H, et al. Perfluorocarbon reduces cell damage from blast injury by inhibiting signal paths of NF-κB, MAPK and Bcl-2/Bax signaling pathway in A549 cells. PLoS One. 2017;12(3):e0173884.

27. Maas DA, Vallès A, Martens GJM. Oxidative stress, prefrontal cortex hypomyelination and cognitive symptoms in schizophrenia. Transl Psychiatry. 2017;7(7):e1171.
28. Zhu Z, Li X, Chen W, Zhao Y, Li H, Qing C, et al. Prenatal stress causes gender-dependent neuronal loss and oxidative stress in rat hippocampus. J Neurosci Res. 2004;78(6):837-44.
29. Altuntas I, Aksoy H, Coskun I, Caykoylu A, Akcay F. Erythrocyte superoxide dismutase and glutathione peroxidase activities, and malondialdehyde and reduced glutathione levels in schizophrenic patients. Clin Chem Lab Med. 2000;38:1277–81.
30. Spiess BD. Perfluorocarbon Emulsions, Platelet Counts, and Inflammation. Shock. 2019;52(1):13-8.
31. Lehmler HJ. Anti-inflammatory effects of perfluorocarbon compounds. Expert Rev Respir Med. 2008;2(2):273-89.
32. Dani C, Costantino ML, Martelli E, Corno C, Fiore GB, Buonocore G, et al. Perfluorocarbons attenuate oxidative lung damage. Pediatr Pulmonol. 2003;36(4):322-9.