Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Dose-dependent Effects of Perfluorocarbon Based Blood Substitute Perftoran on Cardyodinamics in Animal Model of Ischemia-reperfusion Injury

Vladimir Jakovljevic (drvladakgbg@yahoo.com)
University of Kragujevac, Faculty of Medical Sciences, Kragujevac, Serbia, Department of Physiology, University IM Sechenov, 1st Moscow State Medical, Moscow, Russia, Department of Human Pathology https://orcid.org/0000-0002-0071-8376

Sergey Vorobyev
Department of Human Pathology IM Sechenov First Moscow State University

Sergey Bolevich
Department of Human Pathology IM Sechenov First Moscow State Medical University

Elena Morozova
Department of Human Pathology IM Sechenov First State Medical University

Stefani Bolevich
Department of Pathophysiology IM Sechenov First State Medical University

Alexander Saltykov
Department of Human Pathology IM Sechenov First Moscow State Medical University

Peter Litvickiy
Department of Pathophysiology, IM Sechenov First State Medical University

Vladimir Fisenko
Department of Pharmacology, IM Sechenov First State Medical University

Alexander Tsymbal
Department of Pathophysiology, IM Sechenov First State Medical University

Alexandra Orlova
Department of Human Pathology, IM Sechenov Fisrt State Medical University

Tatiana Sinelnikova
Department of Human Pathology IM Sechenov First State Medical University

Maria Kruglova
Department of Human Pathology IM Sechenov First State Medical University

Ekaterina Silina
Department of Human Pathology IM SEchenov First State Medical University

Anastasia Mikhaleva
Department of Human Pathology IM Sechenov First State Medical University

Isidora Milosavljevic
Department of Pharmacy Faculty of Medical Sciences University of Kragujevac

Jovana Jeremic
Department of Pharmacy Faculty of Medical Sciences University of Kragujevac

Tamara Nikolic Turnic
Department of Pharmacy Faculty of Medical Sciences University of Kragujevac

Research Article

Keywords: isolated rat heart, perfluoroemulsion, cardiodynamic parameters, ex vivo-induced ischemic-reperfusion injury

Posted Date: August 12th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-784611/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Abstract

The main goal of this study was to investigate the cardioprotective properties in terms of effects on cardiodynamics of perfluorocarbon emulsion in *ex vivo*-induced ischemic-reperfusion injury of an isolated rat heart. The first part of the study aims to determine the dose of 10% perfluoroemulsion (PFT) that will show the best cardioprotective effect in rats on ex vivo-induced ischemic / reperfusion injury of an isolated rat heart. Depending on whether the animals received saline or PFT, the animals were divided into a control or experimental group, and depending on the application of a dose (8, 12, 16 ml / kg body weight) of saline or PFT. At a dose of 8 ml / kg, the results indicate statistically significantly lower values of the maximum pressure growth rate in the group treated with 10% PFT compared to the control group treated with saline at R5 and R25 points. At a dose of 12 ml / kg, the maximum left ventricular pressure growth rate differed statistically significantly in the PFT group, ie there was an increase in this parameter at points R25 and R30, and the minimum left ventricular pressure growth rate in R15-R30 compared to saline-treated group. At a dose of 16 ml / kg, PFT also had a statistically significant effect on the change in cardiodynamic parameters in an isolated rat heart organ. Based on all the above, we can conclude that Peftoran administered immediately before ischemia (1 hour) has less positive effects on myocardial function in a model of an isolated rat heart compared to earlier administration (10 and 20 hours). Also, the effects of 10% peftoran solution are more pronounced if there is a longer period of time from application to ischemia, ie immediate application of peftoran before ischemia (1 hour) gave the weakest effects on the change of cardiodynamics of isolated rat heart.

1. Introduction

Ischemia denotes a condition of insufficient blood flow with regard to tissue needs, where, in addition to insufficient oxygen supply to the tissue, incomplete removal of metabolites also occurs [1, 2]. Reperfusion is the re-establishment of blood flow after ischemia, and the term ischemic-reperfusion injury means the influence of harmful factors as part of the pathophysiological mechanisms of ischemia and reperfusion. Myocardial ischemia usually occurs due to occlusion of blood vessels. In addition to heart damage that occurs at the time of ischemia, after the re-establishment of blood flow, a number of stressors are produced, which is why myocardial injuries are called ischemic / reperfusion (I /R). Ischemia is a common clinical symptom in CVD, and due to low pH values, reduced oxygen levels and disturbances in K and Ca concentrations, cardiac dysfunction, arrhythmias, myocardial infarction and sudden death can occur [3-5]. On the other hand, damage and reduced myocardial function caused by ischemia can be repaired during reperfusion. However, most often as a result of the activation of a complex inflammatory response in the reperfusion period, irreversible damage occurs, which is called reperfusion injury [6, 7]. Factors that contribute to I / R injuries are very complex and, in addition to those already mentioned, include microvascular dysfunction, the release of reactive oxygen species (ROS), and the activation of mitochondrial apoptosis and necrosis [8, 9].

At the heart of reperfusion injury is endothelial dysfunction. Endothelial dysfunction can be described as an imbalance between the vasodilator and vasoconstrictor products of the endothelium, which is considered to be the core of the systemic pathological process of atherosclerosis and cardiovascular diseases. The endothelium consists of a single layer of endothelial cells that line the inner surface of the vascular lumen, between the blood and vascular smooth muscle cells, but it also forms capillaries and lymph vessels. The endothelium has many vital functions, including the regulation of vascular tone and inflammatory balance [10-12]. Vascular dilatation in response to stress depends in part on a relaxation factor derived from the endothelium, nitric oxide (NO). 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 guanosyl monophosphate, which leads to the relaxation of smooth muscle cells. Loss of endothelium-mediated vasodilator capacity is considered one of the earliest manifestations of cardiovascular damage and precedes the formation of atherosclerotic plaques [13-15].

On the other hand, the main properties of perfluorocarbons, such as the ability to dissolve large amounts of gases and chemical inertness, have enabled the use of perfluoro-organic compounds in biology as gas-carrying and oxygen-carrying

media. In one study, scientists immersed mice in oxygen perfluoro-butyl-tetrahydrofuran (PFBTHF) and they remained alive for some time in a liquid, inhaling oxygen dissolved in a perfluorocarbon medium [16-20]. The same authors attempted to use organo-fluorine compounds as a perfusion medium to supply isolated organs with oxygen. Isolated rat hearts were found to continue to contract intensely when immersed in an oxygen-saturated compound. However, the delivery of electrolytes, glucose, etc. (which are insoluble in perfluoro compounds, such as water) required perfusion with a perfluorocarbon compound to be replaced by perfusion diluted with oxidized blood.

The main goal of this study was to investigate the cardioprotective properties in terms of effects on cardiodynamics of perfluorocarbon emulsion in *ex vivo*-induced ischemic-reperfusion injury of an isolated rat heart.

2. Material And Methods

2.1 Ethical approval

All of the experimental procedures and were carried out in accordance and with the permission of the Institutional ethical committee for the welfare of laboratory animals. Study was approved by the Ethical Committee for the welfare of experimental animals of the Faculty of Medical Sciences, University of Kragujevac, Serbia.

2.2 Experimental protocol

Wistar albino rats were used for this study. Rats were housed in a temperature-controlled vivarium (22 ± 2 ° C), and light and dark cycles alternated at 12 hours. All animals were male, 10 weeks old and average weight 200 ± 20 g. Standard food and water were available to the animals ad libitum. The first part of the study aims to determine the dose of 10% perfluoroemulsion (PFT) that will show the best cardioprotective effect in rats on ex vivo-induced ischemic / reperfusion injury of an isolated rat heart. Depending on whether the animals received saline or PFT, the animals were divided into a control or experimental group, and depending on the application of a dose (8, 12, 16 ml / kg body weight) of saline or PFT, each of the groups was divided into three additional groups:

Control groups:

1. Control (I / R) group - without prior application of solution (n = 6)

2. Control (I / R) group + 0.9% saline at a dose of 8 ml / kg - 1 hour before ischemia (n = 6)

3. Control (I / R) group + 0.9% saline at a dose of 12 ml / kg - 1 hour before ischemia (n = 6)

4. Control (I / R) group + 0.9% saline at a dose of 16 ml / kg - 1 hour before ischemia (n = 6)

Experimental groups

1. Group PFT 8 ml / kg - 1 hour before ischemia (n = 6)

2. Group PFT 12 ml / kg - 1 hour before ischemia (n = 6)

3. Group PFT 16 ml / kg - 1 hour before ischemia (n = 6)

With the exception of the first control group, the remaining control groups, as well as all experimental groups of animals 1 hour before sacrifice and ex vivo induction of ischemic / reperfusion injury of an isolated heart, intraperitoneally treated with a single dose of saline or PFT (8, 12, 16 ml / kg body weight).

Perfluorocarbon based blood substitute-Perftoran

For the purposes of the study, 10% Perftoran was used, which was prepared ex tempore by dissolving equal volumes of 20% Perftoran plus for intravenous administration and standard 0.9% saline.

2.3 Ex vivo Ischemia/Reperfusion Protocol

After short-term anesthesia induced by intraperitoneal administration of a combination of ketamine and xylazine, the animals will sacrifice and from them were isolated hearts.

After placing the sensor in the left ventricle of the heart, a period of stabilization of the heartbeat followed, lasting about half an hour. Establishing stable (correct) heart rate meant that coronary flow after several series of measurements as well as all parameters of cardiac function did not change significantly. The end of the stabilization period marks the beginning of the study of the function of the isolated heart, as well as the examination of the effects of subacute intraperitoneally administered perftoran. After a period of stabilization, the flow of Krebs-Henseleit solution was stopped for 30 minutes, thus subjecting the myocardium to global ischemia. After that, the flow was re-established and all parameters were monitored during a thirty-minute reperfusion (at 1, 5, 10, 15, 20, 25 and 30 minutes). At points marked S (end of stabilization period), as well as at R1, R3, R5, R10, R15, R20, R25 and R30 (minutes of reperfusion), cardiodynamic parameters were recorded and coronary venous effluent was collected for coronary flow analysis and marker measurement. The next parameters were observed: dp / dt max - maximum rate of pressure development in the left ventricle, expressed in mmHg / s, dp / dt min - minimum rate of pressure development in the left ventricle, expressed in mmHg / s, SLVP - systolic pressure in the left ventricle, expressed in mmHg, DLVP - left ventricular diastolic pressure, expressed in mmHg, and HR - heart rate, expressed as heart rate per minute). The parameters of the left ventricular function are monitored and recorded using a computer, which is made possible by the connection between the sensor and the software unit. In addition to the mentioned parameters, blood flow through coronary blood vessels (CF) was monitored. CF is measured by collecting drops of perfusion solution coming out of coronary blood vessels and heart, by fluorometric method, and is expressed in ml / min.

2.4 Statistical analysis

All data are presented as mean plus standard deviations in form of tables. Results are analyzed by descriptive analyses in statistical software IBM SPSS version 26.0.

3. Results

3.1 The effects of a different regimen of 10% PFT at the same dose on cardiodynamics

In the first part of the study, we monitored the effect of a different regimen of 10% peftoran at the same dose on an animal model in relation to the onset of ischemia. Peftoran was administered once 1 hour, 10 hours or 20 hours before the onset of ischemia at the same dose, after which the effects on cardiodynamics were monitored. Applied 1 hour before ischemia, peftoran solution at a dose of 8 ml / kg had a statistically significant effect on individual points in reperfusion in terms of minimal rate of pressure rise, as well as on all points of systolic pressure and coronary flow in terms of reducing these parameters of myocardial function. Applied 1 hour before ischemia, a solution of peftoran at a dose of 12 ml / kg statistically reduced the maximum and minimum rate of pressure increase at the reperfusion endpoints relative to the untreated group, while systolic and diastolic pressure and coronary flow were significantly reduced at all points of reperfusion when peftoran was administered 1 hour before ischemia at a dose of 12 ml / kg. Applied 1 hour before ischemia, peftoran solution at a dose of 16 ml / kg affected the beginning and end of repefusion in terms of maximum growth rate, systolic and diastolic pressure in the first part of repefusion, while heart rate and coronary flow were perfluorinated at the mentioned dose. significantly reduced the values of these parameters at all points of interest during reperfusion (Tables 1 and 2).

Applied 10 hours before ischemia, peftoran solution at a dose of 16 ml / kg significantly affected all points of reperfusion in terms of increased contractility, increased systolic pressure at the beginning of reperfusion and a drastic reduction in

coronary flow.

Finally, peftoran administered 20 hours before ischemia at a dose of 16 ml / kg had a statistically significant effect by increasing the contractility of the isolated rat heart during reperfusion, as well as systolic and diastolic pressure in the left ventricle, and reducing coronary flow. There was no effect on heart rate (Tables 1 and 2).

Definitely, the effects of 10% peftoran solution are more pronounced if there is a longer period of time from application to ischemia, ie immediate application of peftoran before ischemia (1 hour) gave the weakest effects on the change of cardiodynamics of isolated rat heart.

3.2 The effects of a different doses of 10% PFT on cardiodynamics

In the second part of the study, we examined the effect of 10% peftoran on the cardiodynamic parameters of an isolated rat heart using different doses (8, 12 and 16 ml / kg) observed by comparing all experimental groups.

At a dose of 8 ml / kg, the results indicate statistically significantly lower values of the maximum pressure growth rate in the group treated with 10% PFT compared to the control group treated with saline at R5 and R25 points. On the other hand, other parameters such as the minimum rate of pressure rise and heart rate were not significantly changed. Systolic and diastolic pressure in the left ventricle were statistically significantly elevated at all points of reperfusion (R1-R30) in the PFT group compared to the control group treated with saline. Interestingly, coronary flow was statistically significantly reduced at all points of reperfusion (R1-R30) in the PFT group compared to the untreated control group (I / R group) (Tables 1 and 2).

At a dose of 12 ml / kg, the maximum left ventricular pressure growth rate differed statistically significantly in the PFT group, ie there was an increase in this parameter at points R25 and R30, and the minimum left ventricular pressure growth rate in R15-R30 compared to saline-treated group. Then, systolic and diastolic pressure in the left ventricle differed statistically significantly in the PFT group, ie there was an increase in this parameter at points R1-R30 in relation to the group treated with saline and a decrease in relation to the I / R group. Heart rate differed statistically significantly in the PFT group, ie there was an increase in this parameter at points R5-R10 compared to the group treated with saline, as well as coronary flow that was statistically significantly different in the PFT group, ie there was to a decrease in this parameter at points R1-R30 relative to the saline-treated group (Tables 1 and 2).

At a dose of 16 ml / kg, PFT also had a statistically significant effect on the change in cardiodynamic parameters in an isolated rat heart organ. The maximum, minimum left ventricular pressure growth rate as well as systolic and diastolic pressure differed statistically significantly in all groups compared to the PFT 1 hour back group at points R15 – R30. Left ventricular heart rate was statistically significantly different at points R5 and R10 at PFT 10h back compared to the I / R group and coronary flow was statistically significantly different at points R5 to R30 at PFT 20h back compared to all other groups with the same dose. Thus, the dose difference in terms of the effect reflected and in the difference in the applied volume was most impressive by comparing the lowest and highest doses of perftoran, ie 8 and 16 ml / kg (Tables 1 and 2).

Table 1. The mean values of all parameters of cardiodynamics for all tested groups of animals, Results are presented as mean and standard deviations.

Group		Dp/dtmax		Dp/dtmin		SLVP		DLVP		HR		CF	
Control group (I/R group)	S	1361.5	2.1	-991.5	2.1	36.0	1.4	2.4	0.2	304.0	1.4	28.5	0.7
	R1	2033.0	1.4	-842.5	0.7	66.0	2.8	3.5	0.1	251.5	0.7	28.3	0.1
	R5	1677.5	2.1	-1295.0	2.8	62.0	2.8	5.8	0.2	314.5	6.4	29.4	0.3
	R10	1984.5	6.4	-1454.0	1.4	63.5	0.7	4.5	0.6	304.5	6.4	27.4	0.3
	R15	2056.5	3.5	-1476.5	3.5	56.5	2.1	4.2	0.1	298.0	1.4	27.1	0.1
	R20	2066.0	4.2	-1512.0	2.8	54.5	0.7	3.5	0.5	296.5	3.5	24.4	0.3
	R25	2167.5	2.1	-1547.0	2.8	53.0	2.8	3.4	0.4	296.5	3.5	22.5	0.4
	R30	2118.5	0.7	-1363.0	2.8	46.5	2.1	2.4	0.5	285.5	4.9	22.3	0.1
0.9% NaCl 1h before (8ml/kg)	S	2075.0	5.7	-1414.5	6.4	46.0	4.2	3.9	0.1	316.5	3.5	11.4	0.3
	R1	2258.0	1.4	-816.5	3.5	47.5	2.1	2.5	0.6	168.5	0.7	10.2	0.3
	R5	2076.0	4.2	-1128.0	1.4	41.7	0.1	3.9	0.1	346.5	0.7	11.4	0.3
	R10	1908.0	1.4	-1258.0	1.4	41.5	2.1	3.6	0.5	284.5	6.4	11.3	0.1
	R15	1886.0	4.2	-1326.5	3.5	38.5	0.7	3.5	0.6	276.0	4.2	10.2	0.3
	R20	2954.5	6.4	-1917.5	2.1	47.5	2.1	1.6	0.4	285.0	5.7	10.1	0.1
	R25	1858.0	1.4	-1206.5	3.5	35.0	1.4	2.5	0.4	254.5	6.4	11.1	0.1
	R30	2014.5	6.4	-1207.5	2.1	35.0	1.4	2.5	0.4	264.5	6.4	10.1	0.1
0.9% NaCl 1h before (12ml/kg)	S	1718.5	0.7	-966.5	3.5	35.4	0.6	3.5	0.1	277.0	2.8	11.3	0.1
	R1	1765.0	5.7	-746.5	3.5	35.3	1.1	1.8	0.2	258.0	1.4	10.1	0.1
	R5	1888.5	0.7	-1306.0	4.2	43.0	1.4	3.3	0.1	225.5	4.9	11.7	0.1
	R10	2088.5	0.7	-1365.0	5.7	42.5	0.7	2.7	0.3	255.0	5.7	10.9	0.1
	R15	1966.0	4.2	-1156.0	4.2	33.5	2.1	2.4	0.2	268.5	0.7	11.4	0.3
	R20	1854.5	6.4	-1304.5	6.4	33.0	1.4	2.4	0.0	308.0	1.4	10.4	0.1
	R25	1547.0	2.8	-1215.5	4.9	31.0	1.4	1.8	0.1	298.0	1.4	10.9	0.1
	R30	1456.0	4.2	-1215.0	5.7	29.0	0.0	1.7	0.2	308.5	0.7	11.3	0.1
0.9% NaCl 1h before (16ml/kg)	S	1670.5	0.7	-1410.5	0.7	43.3	0.2	2.7	0.0	248.5	0.7	15.3	0.1
	R1	1704.0	4.2	-783.5	3.5	44.4	0.5	2.6	0.1	162.9	3.0	15.3	0.1
	R5	1684.0	4.2	-1054.5	4.9	45.1	0.0	2.6	0.0	253.8	4.6	14.6	0.3
	R10	1834.0	4.2	-1382.0	1.4	48.2	0.1	3.3	0.1	253.6	3.5	14.6	0.3
	R15	1861.5	0.7	-1423.5	3.5	47.5	0.7	3.2	0.1	238.5	0.7	14.1	0.1
	R20	1941.5	0.7	-1494.5	4.9	47.1	1.5	3.4	0.1	236.0	2.8	14.1	0.1

	R25	1872.5	2.1	-1420.5	0.7	48.4	0.9	2.5	0.3	244.0	2.8	13.9	0.1
	R30	1972.5	2.1	-1538.5	0.7	45.5	0.7	2.9	0.1	233.0	1.4	13.7	0.1
0.9% NaCl 10 hours before (16ml/kg)	S	1778.0	1.4	-1113.5	2.1	45.5	2.1	3.3	0.1	237.5	2.1	11.5	0.1
	R1	2658.5	0.7	-1087.0	2.8	82.5	2.1	4.5	0.2	245.0	5.7	11.4	0.3
	R5	2466.5	3.5	-1074.5	0.7	74.5	0.7	4.2	0.2	275.0	5.7	11.6	0.3
	R10	2456.5	3.5	-1572.5	3.5	64.5	0.7	3.8	0.0	282.5	2.1	12.5	0.1
	R15	2585.0	5.7	-1633.0	2.8	56.5	3.5	3.4	0.2	282.0	1.4	12.1	0.1
	R20	2485.0	5.7	-1537.0	2.8	55.0	1.4	2.9	0.1	282.0	1.4	12.1	0.1
	R25	2576.5	3.5	-1506.5	2.1	54.5	0.7	2.6	0.5	260.5	0.7	12.5	0.1
	R30	2365.5	4.9	-1306.5	2.1	46.0	4.2	2.6	0.5	270.5	0.7	12.5	0.1
0.9% NaCl 20 hours before (16ml/kg)	S	2338.0	1.4	-1576.0	1.4	64.0	2.8	5.5	0.7	285.0	5.7	8.5	0.1
	R1	2358.5	0.7	-1203.0	2.8	66.5	0.7	7.6	0.2	285.0	5.7	9.5	0.4
	R5	2306.0	4.2	-1564.0	1.4	76.5	0.7	7.5	0.5	283.0	2.8	10.6	0.3
	R10	2728.0	1.4	-1907.0	2.8	76.4	2.0	8.5	0.6	295.0	5.7	10.1	0.1
	R15	2058.5	0.7	-1814.0	1.4	73.0	2.8	7.9	0.1	294.5	3.5	9.7	0.1
	R20	2495.5	4.9	-1653.5	2.1	70.6	0.1	6.5	0.5	265.5	3.5	9.5	0.1
	R25	2398.5	0.7	-1544.5	0.7	66.7	0.4	6.5	0.5	265.0	4.2	8.4	0.3
	R30	2397.0	2.8	-1603.0	2.8	62.5	0.4	6.4	0.4	266.0	2.8	8.1	0.1
PFT 1 hour before (8ml/kg)	S	1408.0	73.5	-1000.5	31.8	36.0	1.4	2.5	0.1	299.5	6.4	10.6	0.8
	R1	2127.5	135.1	-833.5	16.3	66.0	2.8	3.6	0.2	258.0	5.7	10.0	0.0
	R5	1739.0	70.7	-1306.0	55.2	62.0	2.8	5.5	0.5	305.0	7.1	10.4	0.8
	R10	2060.5	57.3	-1511.5	78.5	63.5	0.7	4.5	0.2	308.5	2.1	10.0	0.0
	R15	2078.0	62.2	-1676.5	154.9	56.5	2.1	4.4	0.1	291.0	5.7	12.0	0.0
	R20	2171.5	71.4	-1533.0	108.9	54.5	0.7	3.7	0.2	292.5	12.0	12.0	0.0
	R25	2272.5	71.4	-1603.5	101.1	53.0	2.8	3.6	0.1	296.0	17.0	10.0	2.8
	R30	2289.5	78.5	-1310.5	92.6	45.1	0.1	2.4	0.3	287.5	17.7	10.5	2.1
PFT 1 hour before (12ml/kg)	S	1536.7	452.6	-1220.0	428.3	43.1	12.7	4.4	2.0	296.0	15.6	10.9	1.0
	R1	2237.7	861.6	-1102.3	404.4	61.6	21.7	6.1	2.0	282.8	27.0	9.9	0.8
	R5	1589.0	479.0	-1034.0	234.9	44.7	7.3	5.5	3.4	295.5	15.6	11.5	1.2

	R10	1874.3	493.2	-1446.0	486.3	47.6	15.3	4.2	3.2	287.0	25.2	11.3	0.9
	R15	1936.7	355.3	-1436.0	399.4	44.5	15.8	3.5	2.6	293.0	8.7	11.3	0.8
	R20	2037.3	392.2	-1432.7	281.7	44.0	13.7	3.8	2.8	296.7	7.8	11.3	0.6
	R25	2080.3	435.9	-1478.0	407.9	45.8	13.9	3.8	2.9	296.7	11.6	11.3	0.9
	R30	2161.3	318.1	-1437.3	290.7	44.6	13.3	3.5	2.8	295.0	7.9	12.0	1.2
PFT 1 hour before (16ml/kg)	S	1883.6	611.9	-1279.7	346.9	55.0	22.1	2.9	0.5	302.7	28.5	15.5	7.4
	R1	2061.7	757.0	-1039.0	350.2	61.0	25.1	5.8	1.0	274.0	118.9	14.0	6.9
	R5	2042.7	431.9	-1380.3	313.6	61.0	19.3	5.1	0.7	297.3	67.1	15.2	7.3
	R10	2342.0	538.7	-1697.7	393.8	64.3	19.1	4.3	0.9	281.7	50.5	14.8	7.6
	R15	2614.0	791.7	-1839.7	508.5	63.8	19.8	4.0	0.7	281.7	35.6	14.9	6.1
	R20	2704.3	839.6	-1588.3	511.2	59.8	17.6	3.9	0.8	285.3	27.2	15.0	5.9
	R25	2739.3	811.8	-1784.7	477.8	57.8	16.4	3.6	0.7	285.7	23.5	14.9	5.5
	R30	2909.0	890.8	-1691.3	456.6	55.4	14.2	3.4	0.8	283.0	18.5	15.1	5.3
PFT 10 hours before (16ml/kg)	S	1040.7	447.8	-701.0	317.8	31.7	15.1	3.9	0.7	263.7	35.4	13.2	2.3
	R1	1834.7	717.9	-1022.0	394.9	60.1	30.1	5.1	2.9	243.3	36.2	13.7	0.8
	R5	1652.3	440.3	-1278.0	551.9	56.1	23.6	4.2	2.2	237.7	37.2	11.4	1.6
	R10	1410.7	357.0	-988.7	321.6	44.3	12.5	2.9	0.0	237.0	40.4	10.5	3.6
	R15	1554.3	486.5	-991.7	395.5	40.2	11.3	2.3	0.8	255.0	31.0	10.7	3.9
	R20	1605.3	328.4	-1022.7	262.6	39.3	6.8	1.5	1.1	247.0	33.6	10.5	4.1
	R25	1743.0	312.2	-1107.0	227.7	39.2	7.5	2.1	0.6	258.3	40.4	10.1	3.8
	R30	1802.7	269.5	-1530.7	1082.7	57.3	35.5	2.0	0.7	257.3	29.4	10.1	3.8
PFT 20 hours before (16ml/kg)	S	1657.7	255.7	-1201.0	252.3	43.2	3.0	3.0	0.3	261.3	27.5	10.9	2.7
	R1	1427.7	487.4	-676.3	252.7	37.9	13.2	3.3	1.2	237.7	24.7	9.9	2.4
	R5	1484.0	401.0	-941.3	398.4	37.6	16.0	2.9	0.9	264.0	8.9	11.7	3.1
	R10	1546.7	474.9	-1026.3	364.1	43.5	17.3	1.8	0.4	269.7	6.8	11.9	3.4
	R15	1561.3	448.1	-1031.3	445.4	34.2	11.8	2.0	0.6	273.0	9.6	11.5	3.3
	R20	1542.3	452.1	-1041.7	363.2	32.4	9.6	1.9	0.8	272.0	14.8	11.4	3.2
	R25	1634.0	552.0	-954.0	243.8	30.2	8.7	1.4	1.5	272.0	22.6	11.4	3.2
	R30	1769.7	331.3	-941.7	226.0	36.6	8.7	1.6	1.6	279.7	18.2	11.5	3.1

Table 2. Differences between selected point of reperfusion period (R1-R30) and stabilization (S) for all parameters and groups separately. Differences are presented as percent (%) of reducing or increasing at one point.

Groups		Dp/dtmax	Dp/dtmin	SLVP	DLVP	HR	CF
Control group (I/R group)	P1	49.3	-15.0	83.3	48.5	-17.3	-0.7
	P5	23.2	30.6	72.2	144.3	3.5	3.2
	P10	45.8	46.6	76.4	89.9	0.2	-3.9
	P15	51.0	48.9	56.9	77.2	-2.0	-4.9
	P20	51.7	52.5	51.4	45.6	-2.5	-14.4
	P25	59.2	56.0	47.2	41.4	-2.5	-21.1
	P30	55.6	37.5	29.2	2.3	-6.1	-21.8
0.9% NaCl 1h before (8ml/kg)	P1	8.8	-42.3	3.3	-35.1	-46.8	-10.5
	P5	0.0	-20.3	-9.4	0.0	9.5	0.0
	P10	-8.0	-11.1	-9.8	-7.0	-10.1	-0.9
	P15	-9.1	-6.2	-16.3	-9.9	-12.8	-10.5
	P20	42.4	35.6	3.3	-59.7	-10.0	-11.4
	P25	-10.5	-14.7	-23.9	-35.5	-19.6	-2.6
	P30	-2.9	-14.6	-23.9	-34.4	-16.4	-11.4
0.9% NaCl 1 h before (12ml/kg)	P1	2.7	-22.8	-0.4	-50.0	-6.9	-10.6
	P5	9.9	35.1	21.5	-5.7	-18.6	3.5
	P10	21.5	41.2	20.1	-21.7	-7.9	-4.0
	P15	14.4	19.6	-5.4	-30.7	-3.1	0.9
	P20	7.9	35.0	-6.8	-32.4	11.2	-8.4
	P25	-10.0	25.8	-12.4	-48.0	7.6	-4.0
	P30	-5.9	0.0	-6.5	-9.1	3.5	4.1
0.9% NaCl 1h before (16ml/kg)	P1	2.0	-44.5	2.8	-2.1	-34.4	0.0
	P5	0.8	-25.2	4.4	-3.2	2.1	-4.6
	P10	9.8	-2.0	11.4	21.5	2.0	-4.6
	P15	11.4	0.9	9.8	21.1	-4.0	-7.8
	P20	16.2	6.0	8.9	25.2	-5.0	-7.8
	P25	12.1	0.7	11.8	-6.5	-1.8	-9.2
	P30	18.1	9.1	5.2	6.5	-6.2	-10.5
0.9% NaCl 10 h before (16ml/kg)	P1	49.5	-2.4	81.3	34.8	3.2	-0.9
	P5	38.7	-3.5	63.7	27.0	15.8	0.9
	P10	38.2	41.2	41.8	14.8	18.9	8.7
	P15	45.4	46.7	24.2	4.2	18.7	5.2
	P20	39.8	38.0	20.9	-13.3	18.7	5.2

	P25	44.9	35.3	19.8	-22.6	9.7	8.7
	P30	-8.2	-13.3	-15.6	0.2	3.8	0.0
0.9% NaCl 20h before (16ml/kg)	P1	0.9	-23.7	3.9	37.3	0.0	11.8
	P5	-1.4	-0.8	19.5	37.1	-0.7	24.7
	P10	16.7	21.0	19.4	54.5	3.5	18.8
	P15	-12.0	15.1	14.1	42.7	3.3	14.1
	P20	6.7	4.9	10.3	17.3	-6.8	11.8
	P25	2.6	-2.0	4.1	17.3	-7.0	-1.2
	P30	2.5	1.7	-2.3	16.6	-6.7	-4.7
PFT 1h before (8ml/kg)	P1	51.1	-16.7	83.3	46.1	-13.9	-5.7
	P5	23.5	30.5	72.2	122.4	1.8	-1.9
	P10	46.3	51.1	76.4	81.6	3.0	-5.7
	P15	47.6	67.6	56.9	79.6	-2.8	13.2
	P20	54.2	53.2	51.4	49.0	-2.3	13.2
	P25	61.4	60.3	47.2	44.9	-1.2	-5.7
	P30	62.6	31.0	25.1	-2.0	-4.0	-0.9
PFT 1h before (12ml/kg)	P1	45.6	-9.6	42.9	36.8	-4.4	-9.2
	P5	3.4	-15.2	3.7	23.2	-0.2	6.1
	P10	22.0	18.5	10.3	-5.7	-3.0	3.7
	P15	26.0	17.7	3.1	-20.7	-1.0	4.3
	P20	32.6	17.4	2.0	-15.0	0.2	3.7
	P25	35.4	21.1	6.1	-13.5	0.2	3.7
	P30	40.7	17.8	3.3	-20.3	-0.3	10.4
PFT 1h before (16ml/kg)	P1	9.5	-18.8	10.9	101.2	-9.5	-9.5
	P5	8.4	7.9	10.9	77.9	-1.8	-1.7
	P10	24.3	32.7	17.0	51.2	-6.9	-4.3
	P15	38.8	43.8	16.1	40.7	-6.9	-3.4
	P20	43.6	24.1	8.8	37.4	-5.7	-3.0
	P25	45.4	39.5	5.1	24.5	-5.6	-3.9
	P30	54.4	32.2	0.8	18.7	-6.5	-2.2
PFT 10h before (16ml/kg)	P1	76.3	45.8	89.7	28.3	-7.7	3.5
	P5	58.8	82.3	77.0	5.7	-9.9	-13.6
	P10	35.6	41.0	40.0	-25.5	-10.1	-20.2
	P15	49.4	41.5	26.8	-41.6	-3.3	-18.7
	P20	54.3	45.9	24.2	-61.1	-6.3	-20.2

	P25	67.5	57.9	23.9	-46.6	-2.0	-23.2
	P30	73.2	118.4	80.9	-48.6	-2.4	-23.2
PFT 20 h before (16ml/kg)	C	-13.9	-43.7	-12.4	8.8	-9.1	-8.6
	P1	-13.9	-43.7	-12.4	8.8	-9.1	-8.6
	P5	-10.5	-21.6	-13.0	-3.2	1.0	8.0
	P10	-6.7	-14.5	0.5	-39.7	3.2	9.2
	P15	-5.8	-14.1	-20.8	-31.5	4.5	6.1
	P20	-7.0	-13.3	-25.0	-37.2	4.1	4.9
	P25	-1.4	-20.6	-30.0	-52.6	4.1	4.9
	P30	6.8	-21.6	-15.3	-45.4	7.0	6.1

4. Discussion

The main purpose of this experimental study was to investigate the cardioprotective properties of perfluorocarbon emulsion in ex vivo-induced ischemic-reperfusion injury of an isolated rat heart.

Peftoran itself as a 10% emulsion has been extensively researched from the moment of its first synthesis back in 1996 until today [22-25]. Perftoran is primarily synthesized as a means that will enable improved transport and delivery of oxygen. Perftoran is given by intravenous infusion in doses of 2-30 ml / kg body weight. Perfluoroan formulated so that the concentration of total perfluorocarbon is 20% wt / vol (10% by volume) can dissolve 69 ml of oxygen in 100 ml of solution at PO2 of 760 mmHg [26]. It is important to note that oxygen dissolves in perfluorocarbon, but is not chemically bound to it, unlike hemoglobin, and diffuses easily through plasma and tissue to areas where reduced oxygen concentrations are present. Because there is a linear relationship between oxygen partial pressure and oxygen content in perfluorocarbon solution, increasing inhaled oxygen can significantly improve oxygen delivery, but this must be done carefully to alleviate oxygen toxicity in the lungs [27]. In cases of massive bleeding, Perftoran is often used with 40-90% oxygen during the acute resuscitation phase to increase the delivery of oxygen from the lungs to the tissue. Perftoran is less concentrated than some other perfluorocarbon emulsions, resulting in a more modest contribution to absolute oxygen carrying capacity (depending on the dose administered), but has been shown to improve tissue oxygenation in multiple animal and human studies with or without supplemental oxygen [28].

Also, perfluorocarbon emulsions serve as a molecular bridge to enhance the movement of gases through the plasma and thus facilitate the delivery of oxygen carried by erythrocytes and it is hypothesized that this facilitation of oxygen movement may be more important than the additional oxygen carried by perfluorocarbons [29].

Perftoran increases the transport and delivery of oxygen, and at the same time facilitates the diffusion of oxygen to the ischemic tissue. The magnitude of its effect depends on many variables, such as plasma perfluorocarbon emulsion concentration, inhaled oxygen fraction, hemoglobin concentration, tissue perfusion, and tissue oxygen level. Animal studies have shown that large mammals can survive via perfluorocarbon emulsions even though they have very low hemoglobin levels, which supports the use of perfluorocarbon emulsions to increase oxygen delivery in hemorrhagic anemia. However, it should be noted that perfluorocarbon emulsions are not “artificial blood” because they lack the ability to provide other basic blood functions, such as coagulation, immune response, and electrolyte maintenance and osmolar homeostasis [30]. They can provide a temporary increase in oxygen supply and reduce the need for blood products.

Preclinical studies support the non-toxicity of peftoran in a rat, mouse, rabbit, and dog model and the mean lethal dose is 239 ml / kg for an adult mouse and one-third lower for young mice. The intravenous lethal dose for rats is 140 ml / kg. Therefore, there is no evidence from preclinical studies on the toxic effects of peftoran, no changes in body weight, blood count or individual organs in terms of negative effects. However, short-term administration (3 days) of a larger volume of peftoran emulsion in some studies led to a transient increase in urine concentration, transaminases, and enlargement of the spleen. Such changes are described as transient and disappear within a period of six months. Literature data show that the elimination half-life of this emulsion is about 90 days, so it is logical where such systemic changes in that period came from. Precisely because of these characteristics and others such as low concentration and low oxygen binder capacity (6.9 ml / dl at PO2 760 mmHg), peftoran emulsion can have a positive effect on the cardiovascular system and body performance in general. Also, studies have shown that infusion of peftoran normalizes mean arterial blood pressure in rats (from 150/70 to 50/30) better than saline solutions that are in clinical use [31-35]. The use of peftoran would be especially important in hemorrhagic shock, bleeding, and in all conditions in which rapid oxygen replacement and blood and oxygen supply to vital organs are required. During our research, we noticed that the emulsion of peftoran has a strong effect by increasing the bioavailability of nitrogen monoxide, which is a powerful vasodilator, but also by increasing the contractile ability of the myocardium of rats. The lipophilic structure of peftoran comes to the fore precisely during the earlier application of peftoran, ie we noticed that the application for example 20 hours before ischemia is more effective in relation to the application immediately before cardiac ischemia [36, 37].

Clinical studies indicate different results regarding the effects of peftoran emulsion. Pharmacodynamics depend exclusively on the dose, condition and disease of the patient, method of administration, etc. and adverse effects occur in about 8% of all patients, such as transient hyperemia, tremor and sometimes hypotension, and very rarely renal colic and pulmonary complications [38, 39].

Overall, the literature data indicate that the efficacy of peftoran and a positive effect were observed in 86.3% of all subjects, and negative effects in 3.3%, and the absence of all effects in 8.3% of subjects. The most common indication is bleeding due to which a peftoran emulsion was applied [40-45].

Taking into account the results of preclinical and clinical studies, as well as the results of our research, it is clear that peftoran emulsion does not have to be only a blood substitute but also a treatment of choice in polytraumas, various shock states, injuries to vital organs of kidneys, brain and heart, but also a powerful tool. to eliminate swelling after craniocerebral trauma. Also, the use of properly dosed perftoran may be the treatment of choice in all conditions of organic dysfunction in adults. Since ischemic diseases of the cardiovascular system basically have reduced bioavailability of vasodilators of nitrogen monoxide and blood supply, it is quite logical to use short-term peftoran which will stimulate the production of nitrogen monoxide in the endothelium and improve vascular function of coronary blood vessels. The future of peftoran use lies in all of the above, in new indications and methods of application, because another name for peftoran can be an antihypoxic and antiischemic blood substitute with mild membranotropic effects.

Conclusion

Based on all the above, we can conclude that Peftoran administered immediately before ischemia (1 hour) has less positive effects on myocardial function in a model of an isolated rat heart compared to earlier administration (10 and 20 hours). Also, the effects of 10% peftoran solution are more pronounced if there is a longer period of time from application to ischemia, ie immediate application of peftoran before ischemia (1 hour) gave the weakest effects on the change of cardiodynamics of isolated rat heart. The future of peftoran use is in new indications and methods of application and another name for peftoran can be antihypoxic and antiischemic blood substitute with mild membranotropic effects.

Declarations

Funding: NA

Conflicts of interest/Competing interests: The authors declare no conflict of interest.

Availability of data and material (data transparency): Data are available from the correspondence author upon reasonable request.

Code availability (software application or custom code): NA

Ethics approval (include appropriate approvals or waivers): Study is approved by the Ethical Committee for the welfare of experimental animals of the Faculty of Medical Sciences, University of Kragujevac, Serbia.

Consent to participate (include appropriate statements): NA

Consent for publication (include appropriate statements): NA

Authors' Contribution:

VLJ; SV: Conceptualization, Writing- Original draft preparation; SV; SB; EM; SSB; AS; PL; Validation, Visualization, and Methodology. VF; AT: Methodology. JJ; IS; TNT: Software. AO; TS: Data curation; Visualization. JJ; VLJ: Investigation. MK; ES; AM; TNT: Conceptualization, Writing-Original draft preparation, Supervision, and Writing-Reviewing and Editing.

Acknowledgments: This research was supported by the Faculty of Medical Sciences, University of Kragujevac.

References

1. Spahn D (1999) Blood Substitutes Artificial oxygen carriers: perfluorocarbon emulsions. Critical Care 5:R93-R97. https://doi.org/10.1186/cc364
2. Bilenko MV (1989) Ischemic and reperfusion injures of organs molecular mechanisms, ways of preventing and treatment. Moscow: Medicine *in Russian*
3. Blaisdell FW (1989) The reperfusion syndrome. Microcirc Endothelium Lymphatics 5:127–141.
4. Ar'Rajab A, Dawidson I, Fabia R (1996) Reperfusion injury. New Horiz 4:224–234.
5. Lindsay TF, Luo XP, Lehotay DC, Rubin BB, Anderson M, Walker PM, Romaschin AD (1999) Ruptured abdominal aortic aneurysm, a "two-hit" ischemia/reperfusion injury: evidence from an analysis of oxidative products. J Vasc Surg 30:219–228. https://doi.org/10.1016/s0741-5214(99)70131-x
6. Vlasov TD, Smirnov DA, Nutfullina GM (2001) [Adaptation of the rat small intestine to ischemia]. Ross Fiziol Zh Im I M Sechenova 87:118–124. *in Russian*
7. Gennaro M, Mohan C, Ascer E (1996) Perfluorocarbon emulsion prevents eicoasanoid release in skeletal muscle ischemia and reperfusion. Cardiovasc Surg 4:399–404. https://doi.org/10.1016/0967-2109(95)00060-7.
8. Memezawa H, Katayama Y, Shimizu J, Suzuki S, Kashiwagi F, Kamiya T, Terashi A (1990) Effects of fluosol-DA on brain edema, energy metabolites, and tissue oxygen content in acute cerebral ischemia. Adv Neurol 52:109–118.
9. Mosca RS, Rohs TJ, Waterford RR, Childs KF, Brunsting LA, Bolling SF (1996) Perfluorocarbon supplementation and postischemic cardiac function. Surgery 120:197–204. https://doi.org/10.1016/s0039-6060(96)80288-1
10. Kubes P (1993) Ischemia-reperfusion in feline small intestine: a role for nitric oxide. Am J Physiol 264:G143–G149. https://doi.org/10.1152/ajpgi.1993.264.1.G143
11. Mathy-Hartert M, Krafft MP, Deby C, Deby-Dupont G, Meurisse M, Lamy M, Riess JG (1997) Effects of perfluorocarbon emulsions on cultured human endothelial cells. Artif Cells Blood Substit Immobil Biotechnol 25:563–575. https://doi.org/10.3109/10731199709117453
12. Speiss BD (2009) Perfluorocarbon emulsions as a promising technology: a review of tissue and vascular gas dynamics. J Appl Physiol 106:1444-1452. https://doi.org/10.1152/japplphysiol.90995.2008

13. Cohn C, Cushing M (2009) Oxygen Therapeutics: Perfluorocarbons and Blood Substitute Safety. Crit Care Clin 25:399-414. https://doi.org/10.1016/j.ccc.2008.12.007
14. Cabrales P, Ingaglietta M (2013) Blood substitutes: evolution from non-carrying to oxygen and gas carrying fluids. ASAIO J 59(4):337-354. https://doi.org/10.1097/MAT.0b013e318291fbaa
15. Verdin-Vasquez RC, Zepeda-Perez C, Ferra-Ferrer R, Chavez-Negrete A, Contreras F, Barroso-Aranda J (2006) Use of Perftoran Emulsion to Decrease Allogeneic Bllod Transfusion in Cardia Surgery: Clinical Trail. Artif Cells Blood SubstitImmobil and Biotechnol 34:433-454. https://doi.org/10.1080/10731190600683969
16. Eckmann DM, Lomivorotov VN (2003) Microvascular gas embolization clearance following perfluorocarbon administration. J Appl Physiol 94:860-868. https://doi.org/10.1152/japplphysiol.00719.2002
17. Mayevskiy EI, Aksenova OG, Bogdanova LA, Moroz VV, SeninaRYa, Pushkin SYu, Ivanitskiy GR (2001) Analysis of Clinical and Adverse Effects During Phase I and II Clinical Studies of Perftoran. Russian Blood Banking Bulletin No.4
18. Maevsky E, Ivanitsky G, Bogdanova L, Axenova O, Karmen N, Zhiburt E, Senina R, Pushkin S, Maslennokov I, Orlov A (2005) Clincial results of Perftoran application: Present and Future. Artif Cells Blood SubstitImmobil and Biotechnol 33:37-46. https://doi.org/10.1081/bio-200046654
19. Kozhura VL, Basarab DA,Timkina MI,Golubev AM, Reshetnyak VI, Moroz VV (2005) Reperfusion injury after critical intestinal ischemia and its correction with perfluorochemical emulsion “perfloran”: World J Gastroenterol 11:7084-7090. https://doi.org/10.3748/wjg.v11.i45.7084
20. Anthes Anthes E (2015) Evidence-based medicine: Save blood, save lives. Nature 520:24-6. https://doi.org/10.1038/520024a
21. Abutarboush R, Saha BK, Mullah SH, Arnaud FG, Haque A, Aligbe C, Pappas G, Auker CR, McCarron RM, Moon-Massat PF, Scultetus AH (2016) Cerebral Microvascular and Systemic Effects Following Intravenous Administration of the Perfluorocarbon Emulsion Perftoran. J Funct Biomater 7(4):29. https://doi.org/10.3390/jfb7040029
22. Aimo A, Borrelli C, Giannoni A, Pastormerlo LE, Barison A, Mirizzi G, Emdin M, Passino C (2015) Cardioprotection by remote ischemic conditioning: Mechanisms and clinical evidences. World J Cardiol 7:621-632. https://doi.org/10.4330/wjc.v7.i10.621
23. Bryan NS, Calvert JW, Elrod JW, Gundewar S, Ji SY, Lefer DJ (2007) Dietary nitrite supplementation protects against myocardial ischemia-reperfusion injury. Proc Natl Acad Sci USA 104:19144-19149. https://doi.org/10.1073/pnas.0706579104
24. D'ascenzo F, Cavallero E, Moretti C, Omede P, Sciuto F, Rahman IA, Bonser RS, Yunseok J, Wagner R, Freiberger T, Kunst G, Marber MS, Thielmann M, Ji B, Amr YM, Modena MG, Zoccai GB, Sheiban I, Gaita F (2012) Remote ischaemic preconditioning in coronary artery bypass surgery: a meta-analysis. Heart 98:1267-1271. https://doi.org/10.1136/heartjnl-2011-301551
25. Depre C, Vatner SF (2007) Cardioprotection in stunned and hibernating myocardium. Heart Fail Rev. 12:307-317. https://doi.org/10.1007/s10741-007-9040-3
26. Downey JM (1990) Free radicals and their involvement during long-term myocardial ischemia and reperfusion. Annu Rev Physiol 52:487-504. https://doi.org/10.1146/annurev.ph.52.030190.002415
27. Endemann DH, Schiffrin EL (2004) Endothelial dysfunction. J Am Soc Nephrol 15:1983-1992. https://doi.org/10.1097/01.ASN.0000132474.50966.DA
28. Mangoni AA, Sherwood RA, Swift CG, Jackson SH (2002) Folic acid enhances endothelial function and reduces blood pressure in smokers: a randomized controlled trial. J. Int. Med 252:497–503. https://doi.org/10.1046/j.1365-2796.2002.01059.x
29. Lai WK, Kan MY (2015) Homocysteine-Induced Endothelial Dysfunction. Ann Nutri Metab 67:1-12. https://doi.org/10.1159/000437098

30. Jacobsen DW (2000) Hyperhomocysteinemia and oxidative stress: time for a reality check? Arterioscler Thromb Vasc Biol 20:1182-1184. https://doi.org/10.1161/01.atv.20.5.1182
31. Czerska M, Mikołajewska K, Zielinski M, Gromadzinska J, Wasowicz W (2015) Today's oxidative stress markers. Med Pr. 66:393-405. https://doi.org/10.13075/mp.5893.00137
32. Stoian I, Oros A, Moldoveanu E (1996) Apoptosis and free radicals. Biochem Mol Med. 59:93-97. https://doi.org/10.1006/bmme.1996.0072
33. Djukic M, Ninkovic M, Jovanovic M (2008) Oxidative stress: Clinical diagnostic significance. JMB 27:409-425. https://doi.org/10.2478/v10011-008-0024-1
34. Halliwell B (1994) Free radicals, antioxidants, and human diseases: Curiosity, cause, or consequence? Lancet 344: 721–724. https://doi.org/10.1016/s0140-6736(94)92211-x
35. Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S, Dhama K (2014) Oxidative stress, prooxidants, and antioxidants: the interplay. Biomed Res Int. 2014:761264. https://doi.org/10.1155/2014/761264
36. Tak PP, Firestein GS (2001) NF-kappaB: a key role in inflammatory diseases. J Clin Invest 107:7-11. https://doi.org/10.1172/JCI11830
37. Hampton MB, Kettle AJ, Winterbourn CC (1998) Inside the neutrophil phagosome: oxidants, myeloperoxidase, and bacterial killing. Blood 92:3007-3017.
38. Romero MM, Basile JI, Lopez B, Ritacco V, Barrera L, Sasiain Mdel C, Aleman M (2014) Outbreaks of Mycobacterium tuberculosis MDR strains differentially induce neutrophil respiratory burst involving lipid rafts, p38 MAPK and Syk. BMC Infect Dis 14:262. https://doi.org/10.1186/1471-2334-14-262
39. Bae YS, Kang SW, Seo MS, Baines IC, Tekle E, Chock PB, Rhee SG (1997) Epidermal growth factor (EGF)-induced generation of hydrogen peroxide. Role in EGF receptor- mediated tyrosine phosphorylation. J Biol Chem 272:217-221.
40. Chandel NS, Maltepe E, Goldwasser E, Mathieu CE, Simon MC, Schumacker PT (1998) Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. Proc Natl Acad Sci USA 95:11715-11720. https://doi.org/10.1073/pnas.95.20.11715
41. Chandel NS (2014) Mitochondria as signaling organelles. BMC Biol 12:34. https://doi.org/10.1186/1741-7007-12-34
42. Chandel NS, Trzyna WC, McClintock DS, Schumacker PT (2000) Role of oxidants in NF- kappa B activation and TNF-alpha gene transcription induced by hypoxia and endotoxin. J Immunol 165(2):1013-1021. https://doi.org/10.4049/jimmunol.165.2.1013
43. Valko M, Rhodes CJ, Moncol J, Izakovic M (2006) Free radicals metals and antioxidants in oxidative stress¬induced cancer. Chem Biol Interact 160: 1-40. https://doi.org/10.1016/j.cbi.2005.12.009
44. Steinberg D (1997) Low density lipoprotein oxidation and its pathobiological significance. J Biol Chem 272:20963-20966. https://doi.org/10.1074/jbc.272.34.20963
45. Anavi S, Ni Z, Tirosh O, Fedorova M (2014) Steatosis-induced proteins adducts withlipid peroxidation products and nuclear electrophilic stress in hepatocytes. Redox Biol. 4C:158-168. https://doi.org/10.1016/j.redox.2014.12.009