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CORM-2 AFFECTS LEVEL OF MALONDIALDEHYDE AND EXPRESSION OF MYOCARDIAL MARKERS PTGS2, ANP, BNP, MVH7 UNDER INDUCED FERROPTOSIS

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The formation of pro-oxidants in tissues is a dangerous phenomenon for the body. The emergence of pro-oxidants damages cell structures. Myocardium deserves special attention. This increases the level of superoxide radicals, hydrogen peroxide, and hydroxyl radicals under ischemia conditions. The formation of toxic active oxygen forms always accompanies ischemic-reperfusion myocardial damage. The phenomenon of ferroptosis, which comprises the release of iron ions, increases lipid peroxidation to form malondialdehyde (MDA).

The endogenous formation of carbon monoxide, as a consequence of the breakdown of heme-containing proteins, leads to increased expression of heme-oxygenase 1 (HO-1). It is an enzyme with antioxidant and anti-inflammatory properties. Substances that release carbon monoxide after exposure to physiological media are useful for research. Following ingestion, they decay slowly, releasing carbon monoxide. This gas transmitter may have a positive effect on the cells as ferroptosis develops. Doxorubicin (DOX) injected multiple times intraperitoneally into laboratory mice for ferroptosis induction. Every group of animals received additional hemin or HO-1 inhibitor (zinc-protoporphyrin), or donor CO – tricarbonyldichlorothenium (II)-dimer (CORM-2) in doses of 20 and 200 mg/kg. By the end of the experiment, the level of expression of the myocardial markers of Ptgs2 mRNA, Anp, Bnp, and Mvh7 was determined. Simultaneously, the myocardial homogenate determined the content of the MDA.

As a result, the injection of CORM-2 at a concentration of 20 mg/kg found to reduce damage marker expression in the myocardium under conditions of ferroptosis induction. Same CORM-2 concentration also reduced the MDA content in the myocardium. Use of the HO-1 blocker had a positive effect on myocardial ferroptosis. The result showed that excessive activation of HO-1 has a negative effect on the heart. The influence of DOX and hemin on myocardium was negative, with increased expression of Ptgs2, Anp, Bnp, and Mvh7 markers. A high level of myocardial MDA accompanied the increased expression of these markers.

Key words: *CORM-2, carbon monoxide, myocardium, ferroptosis.*

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CORM-2 ВПЛИВАЄ НА РІВЕНЬ МАЛОНОВОГО ДІАЛЬДЕГІДУ ТА ЕКСПРЕСІЮ МІОКАРДІАЛЬНИХ МАРКЕРІВ PTGS2, ANP, BNP, MVH7 ПІД ЧАС ІНДУКЦІЇ ФЕРОПТОЗУ

Утворення прооксидантів у тканинах є небезпечним явищем для організму. Внаслідок утворення прооксидантів ушкоджуються структурні елементи клітини. Особливої уваги заслуговує міокард. В умовах ішемії у міокарді підвищується рівень супероксид-радикалів, перекису водню та гідроксильних радикалів. Ішемічне та реперфузійне ушкодження міокарду супроводжується утворенням токсично активних форм оксигену. Особливої уваги заслуговує явище фероптозу, яке полягає у вивільненні йонів феруму з подальшим посиленням перекисного окиснення ліпідів та утворенням малонового діальдегіду (MDA).

Ендогенне утворення монооксиду карбону, як наслідок розпаду гем-вмісних білків, призводить до посилення експресії гемоксигенази-1. Цей фермент має антиоксидантні та протизапальні властивості. Для дослідження фізіологічного впливу монооксиду карбону використовують молекули-донори. Після їхнього потрапляння до організму вони повільно розпадаються, вивільняючи монооксид карбону (CO). Цей газотрансміттер може мати позитивний вплив на кардіоміоцити під час розвитку фероптозу. Для індукції фероптозу лабораторним мишам декілька разів внутрішньоочеревинно уводили доксорубіцин (DOX). Кожна окрема група отримувала додатково гемін, або інгібітор гемоксигенази-1 (цинк-протопорфірин), або донор CO – CORM-2 у дозах 20 та 200 mg/kg. Наприкінці експерименту визначали рівень експресії mRNA маркерів ушкодження міокарду Ptgs2, Anp, Bnp та Mvh7. Одночасно визначали вміст малонового діальдегіду у гомогенаті міокарду.

Як результат було встановлено, що введення CORM-2 у концентрації 20 mg/kg в умовах індукції фероптозу, знижувало експресію маркерів ушкодження міокарду. Ця концентрація донора CO також знижувала вміст MDA в тканині серця. Застосування блокатора HO-1 мало позитивний ефект в умовах індукованого фероптозу міокарда. Це вказує на негативну дію надмірної активації HO-1. Вплив DOX та геміну на міокард був негативним: посилювалася експресія маркерів Ptgs2, Anp, Bnp та Mvh7. Зазначені зміни супроводжувалися підвищеними показниками рівня малонового діальдегіду.

Ключові слова: *CORM-2, монооксид карбону, міокард, фероптоз.*

Oxygen in the body is reduced to water by oxidative phosphorylation in the mitochondria. Up to 5% of the total amount of oxygen in the body, following enzyme reactions, transforms into active forms which are very toxic to cells. The formation of these pro-oxidants damages proteins, nucleic acids, and cell membrane lipids. The generation of active forms of oxygen is constantly taking place [1].

Myocardial cell damage induced by ischemia/reperfusion cycles may be associated with the forming of toxic active oxygen species such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals [2]. Excess calcium ions can also trigger the formation of active oxygen species. Elevated calcium concentration activates the proteases that transform xanthine dehydrogenase. Xanthin oxidase can use oxygen as an electron acceptor. Hydrogen anion and peroxide can react to form hydroxyl radicals (resulting in tissue injury). Oxygen free radicals play a significant role in tissue damage during ischemia/reperfusion of the myocardium [5].

During myocardial lesions, iron ions are released, and this also stimulates free radical reactions. An additional source of free radicals in the mitochondrial electron transport chain. Free radicals formed in mitochondria can cause point mutations, disrupting the DNA structure of mitochondrial genes. A consequence of damage to the genome of mitochondria is disruption of the tissue respiration process and increased formation of oxygen radicals. Mitochondrial dysfunction and superoxide formation often occur during reperfusion. The increase in the production of reactive forms of oxygen is because of the low anti-oxidant activity of the myocardial tissue. In myocytes, as in endothelial cells, catalase activity is very weak. Ischemic damage produces lipoperoxides compounds comprising unsaturated fatty acid residues with hydroxyl radical or singlet (activated) oxygen. While tissue perfusion is disturbed, the formation of lipoperoxides increases significantly, which also triggers the process of lipid oxidation by free radicals [5]. Equally a result of long and intense physical exertion, hypoxia processes occur [3, 6]. Hypoxia alters the pro-oxidant-antioxidant balance, enhances the oxidation processes of metabolites, and suppresses the antioxidant system [4, 8]. The resulting lipoperoxides can significantly damage the endothelial layer, thus provoking furthermore radical reactions to cell membranes [7]. Simultaneously, the accumulation of free-radical compounds reduces the bioavailability of nitrogen oxide (NO), and the decrease in NO production is a marker of endothelium dysfunction [12].

The weakening of antioxidant protection and uncontrolled enhancement of lipid peroxidation processes are accompanied by the accumulation of lipid peroxidation products, a low-level dialdehyde that destabilizes cell membranes [13].

Especially noteworthy is the onset of ferroptosis, which is observed during myocardial ischemia-reperfusion. An important feature of ferroptosis is iron-dependent lipid peroxidation, which causes oxidative stress and cell death [15].

Recently, in search of ways to minimize the effects of oxidative stress, great attention is paid to the new open gas transmitter – carbon monoxide (CO). Endogenous CO forms in the body through the decomposition of hem-containing proteins. The main part of CO forms during hemoglobin splitting [16]. Recent studies show CO has anti-inflammatory properties, reduces the formation of active oxygen forms, and has vasodilatory properties [7]. Notwithstanding these properties, the problem with the practical application of carbon monoxide is that the amount of CO is difficult to measure and that inhalation is very dangerous. CO donors therefore use. Once ingested, CO donors release this gas slowly and thus see it as a promising drug.

Therefore, further research is needed to determine the effect of donor CO on the oxidative status of the heart muscle and the expression of myocardial damage markers.

MATERIALS AND METHODS

The study was using BALB/10-week-old male laboratory mice. Six experimental animal groups were formed. The first group was thought to be the control group, so the animals received saline in equilibrium. Intraperitoneal injections of doxorubicin (20 mg/kg, DOX, «Actavis», Italy) for animals in five groups were conducted to start ferroptosis in the heart. We made injections on the first and eighth days of the experiment. On the 9th day, anesthetized with pentobarbital (70 mg/kg, i.p) and took blood and heart for further research. Group 2 got only doxorubicin. Group 3 inhibited Hmox1 by injecting protoporphyrin zinc (ZnPP, Sigma, 10 mg/kg, dissolved in 10% DMSO and saline) 1 day before doxorubicin administration. The 4th group would inject with hemin (Sigma, 2.5 mg/ml), which was dissolved in 10% ammonium hydrochloride solution, after which 0.15 M sodium chloride solution was dissolved and intraperitoneally injected for induction of HO-1 (25 mg/kg body weight). CORM-2 was first diluted in the DMSO (in the resulting solution, the DMSO concentration was no higher than 0.1%) and in the saline solution and injected into the animals of the 5th and 6th groups in concentrations of 20 mg/kg and 200 mg/kg, respectively. The type of influence on the core of the injected compounds was assessed by determining the heart expression level of Ptgs2 mRNA, Anp, Bnp, Mvh7 in the myocardium using real-time PCR.

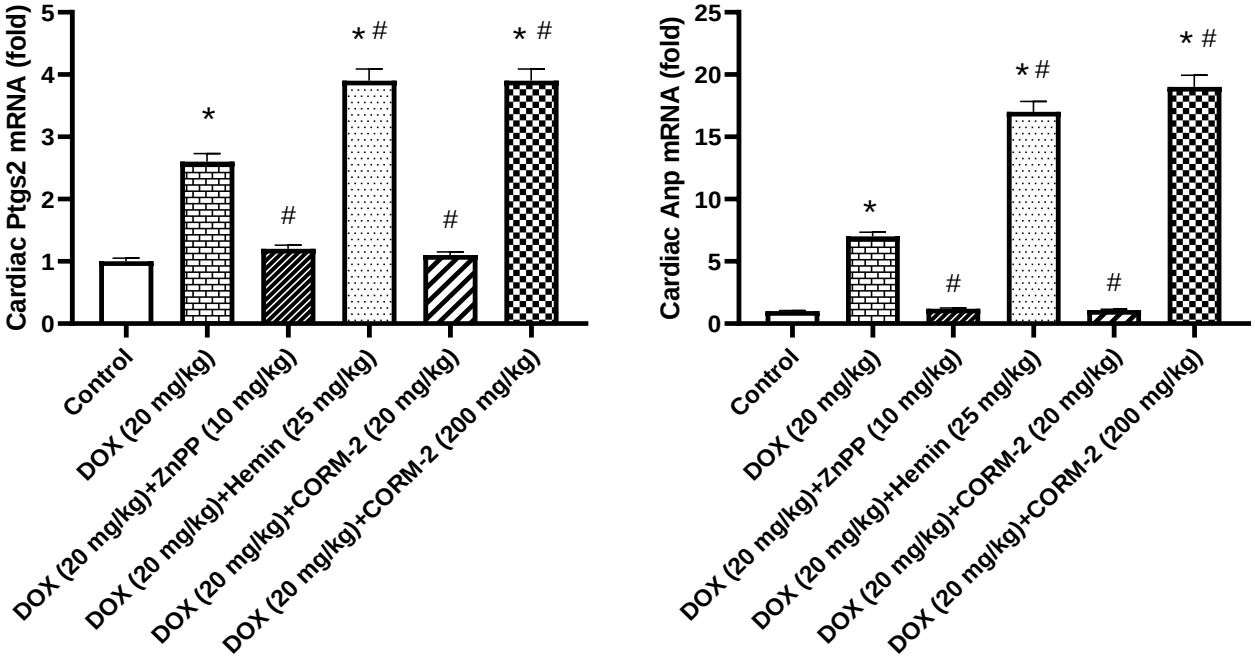
Primers (Invitrogen, Grand Island, NY) used were as follows (5=-3=): mouse Ptgs2: (Forward: 5’- CTGCGCCTTTTCAAGGATGG, Reverse: 5’- GGGGATACACCTCTCCACCA, Gene ID: 19225), mouse Anp: (Forward: 5’- TCGTCTTGGCCTTTTGGCT, Reverse: 5’- TCCAGGTGGTCTAGCAGGTTCT, Gene ID: 230899) mouse Bnp: (Forward: 5’- AAGTCCTAGCCAGTCTCCAGA, Reverse: 5’- GAGCTGTCTCTGGGCCATTTC, Gene ID: 18158) mouse Myh7: (Forward: 5’- GCTGAAAGCAGAAAGAGATTATC, Reverse: 5’- TGGAGTTCTTCTCTTCTGGAG, Gene ID: 140781).

The MDA level was determined by a colored reaction with 2-thiobarbituric acid [9]. Samples were incubated at t=100°C for 15 minutes. It was then cooled to 20-22 C and centrifuged at 3,000 rpm/min for 10 min on a centrifuge CM–6MT (ELMI, Latvia). Solution’s extinction was measured at λ=532 nm. MDA content is calculated in mmol/mg of protein. Amount of protein is determined by the Bradford method.

Statistical analysis of the results was carried out using the «Statistica 10» program. The validity of the differences was determined according to the Man-Whitney and Wilcoxon criteria. Changes were considered likely under $P<0.05$. The research has been carried out under Directive 2010/63/EU on the protection of animals used for scientific purposes.

RESULTS AND DISCUSSION

The induction of ferroptosis by DOX has shown that this compound enhances the expression of Ptgs2 mRNA. It has been found that the intensity of this expression varies depending on the substances injected with DOX. A significant increase in expression was observed in the group receiving DOX with hemin and DOX with CORM-2 at a dose of 200 mg/kg (Fig. 1-A). In the group treated with DOX from ZnPP, as well as DOX from CORM-2 at a dose of 20 mg/kg, the Ptgs2 mRNA expression did not differ from the control group, showing the ability of the corresponding compounds to reduce the negative effects of DOX (fig. 1-A).



A *B*

Fig. 1. ZnPP, hemin, CORM-2 effects in doses of 20 and 200 mg/kg for expression of Ptgs2(A) and Anp(B) in the myocardium of the mice studied groups

*Notes: * P<0.05 compared to control values, #P<0.05 compared to DOX group values*

Similar results were got after determining the activity of heart Anp mRNA: DOX amplifies the expression of this gene, but the injection of DOX with hemin and DOX with CORM-2 at 200 mg/kg increased expression more than just the injection of DOX (more than twice the difference). The injection together with ZnPP, as well as with CORM-2 at a dose of 20 mg/kg, neutralized the negative effects of DOX (fig. 1-B).

In the case of Bnp mRNA, DOX increased the expression of this marker of myocardial damage compared to the control. The injection of DOX with hemin and DOX with CORM-2 at 200 mg/kg increased the expression of Bnp mRNA. ZnPP, as well as CORM-2 at a dose of 20 mg/kg, injected with DOX, inhibited the expression of Bnp mRNA (fig. 2-A).

An important marker of myocardial damage is the increased expression of Mvh7 mRNA. DOX injections with ZnPP and CORM-2 at a dose of 20 mg/kg reduced the expression of this marker. However, in the group that injected DOX with hemin, and in the group that received DOX from CORM-2 at 200 mg/kg, there was an increase in Mvh7 mRNA expression compared to the group receiving DOX (Fig. 2-B).

The determination of the MDA level in the myocardium of the study groups showed that DOX injection leads to an increase in this endogenous aldehyde. Elevated MDA levels were observed in animals injected with DOX with hemin, and in the group given DOX from CORM-2 at

a dose of 200 mg/kg. Using CORM-2 at a dose of 20 mg/kg shows myocardial protection against DOX. A similar effect was observed with DOX and ZnPP injection (fig. 3).

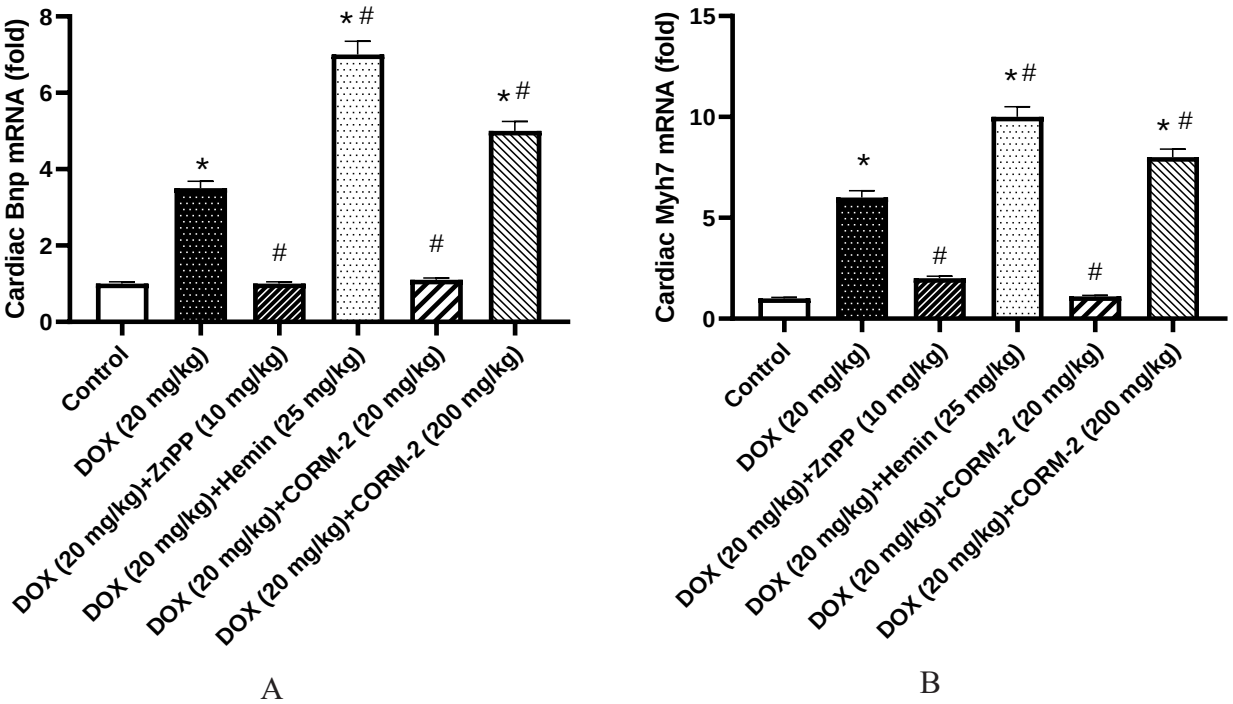


Fig. 2. Effects of ZnPP, hemin, CORM-2 at a dose of 20 and 200 mg/kg per expression of Bnp(A) and Myh7(B) in the myocardium of the mice studied groups

*Notes: * P<0.05 compared to control values, #P<0.05 compared to DOX group values*

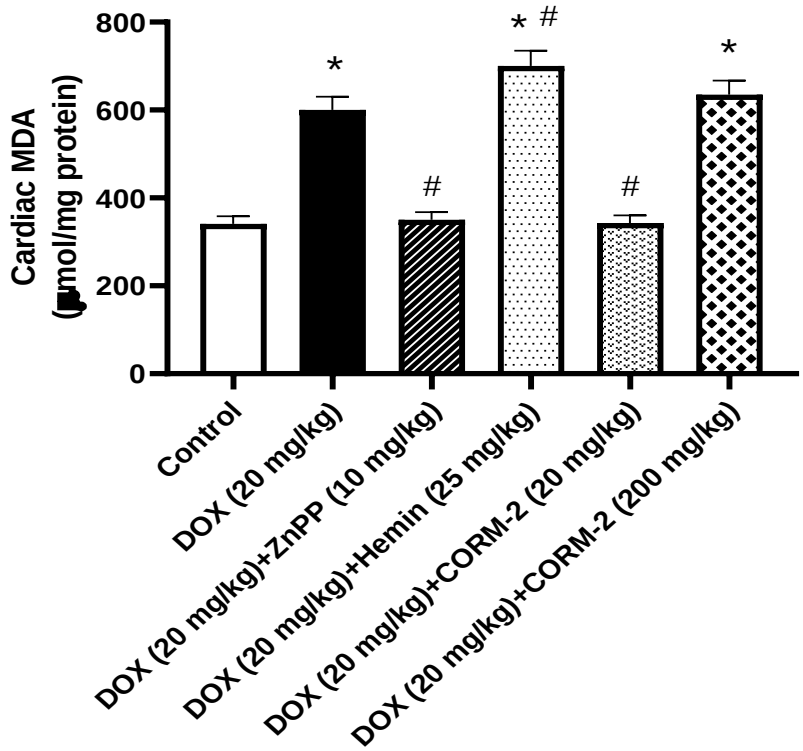


Fig. 3. MDA level in myocardium mice under the influence of ZnPP, hemin, CORM-2 at a dose of 20 and 200 mg/kg under conditions of DOX-induced ferroptosis

*Notes: * P<0.05 compared to control values, #P<0.05 compared to DOX group values*

The results show that high concentrations of CORM-2 can increase oxidative stress and hurts harms the myocardium under conditions of DOX-induced ferroptosis. Injection of high concentrations of hemin has a similar effect. However, the use of CORM-2 in small concentrations, as well as a hem oxygenase blocker, has showed a positive effect on the expression of myocardial damage markers and oxidative stress levels. Their application is promising for reducing the negative effects on myocardial ischemic or reperfusion damage.

Ptgs2, Anp, Bnp, and Mvh7 are sensitive markers of myocardial dysfunction, so it can be argued that excessive stimulation of HO-1 by hemin and CORM-2 in high doses negatively affects the myocardial state. The gene Ptgs2 is responsible for the synthesis of the cyclooxygenase-2 (COX2) enzyme, responsible for inflammatory reactions. COX2 is involved in fatty acid metabolism, lipid metabolism, and prostaglandin biosynthesis. Anp is a polypeptide hormone synthesized by specialized myoendocrine cells of the atrium and plays a significant role in regulating aqueous, salt, and lipid metabolism. Anp is produced by myoendocrine cells in response to increased blood flow into the heart and causes distension of the atrial walls, preventing heart hypertrophy. In response to increased mechanical stress and cardiac sprain, the ventricles of the heart secrete a B-type natriuretic peptide, which protects the heart from the negative effects of overloading.

The results are consistent with the results got from the study of fibroblasts in mice. It has been shown that overexpression of HO-1 leads to apoptosis mediated by tumor necrosis factor-alpha [11]. However, CO is also reported to cause apoptosis, but the antiapoptotic effect may be mediated by endogenous CO. This is consistent with the evidence for the beneficial effects on cardiomyocytes of low CO concentrations [14].

Thus, the gradual release of a small amount of CO from CORM-2 has a positive effect on the myocardium, preventing the development of oxidative stress and myocardial damage. These effects may be because of inhibition of the P450-dependent monooxygenase system in smooth muscle cells [16]. CO released in small quantities has anti-inflammatory properties [10].

CONCLUSIONS

Injection of CORM-2 at a concentration of 20 mg/kg reduced the expression of damage markers in the myocardium under conditions induced by ferroptosis. This concentration of the carbon monoxide donor also reduced the MDA content in the heart tissue. Using an HO-1 blocker in ferroptosis has also shown a positive effect on the myocardium. This confirms our hypothesis about the harmful effects of excessive activation of HO-1. The combined effect of DOX and hemin hurt the myocardium: the expression of Ptgs2, Anp, Bnp, and Mvh7 markers increased. These characteristics were got against the background of the highest level of MDA in the myocardium.

REFERENCES

1. Abrahams S, Haylett WL, Johnson G, Carr JA, Bardien S. Antioxidant effects of curcumin in models of neurodegeneration, aging, oxidative and nitrosative stress: A review. Neuroscience. 2019;406:1-21. https://doi.org/10.1016/j.neuroscience.2019.02.020
2. Beschasnyi SP, Hasiuk OM. The carbon monoxide donor, topiramate, and blockers of aquaporine receptors decrease myocardial ischemia-reperfusion injiry. Fiziologichnyi Zhurnal. 2021;67(5):30-38. https://doi.org/10.15407/fz67.05.030
3. Bloomer RJ, Cole B, Fisher-Wellman KH. Racial differences in postprandial oxidative stress with and without acute exercise. Int. J. Sport Nutr. and Exercise Metabol. 2009;19 (5): 457-472. DOI: https://doi.org/10.1123/ijsnem.19.5.457
4. Bonekamp NA, Völkl A, Fahimi HD, Schrader M. Reactive oxygen species and peroxisomes: struggling for balance. Biofactors. 2009;35(4):346-355. https://doi.org/10.1002/biof.48
5. Girotti AW. Lipid hydroperoxide generation, turnover, and effectors action in biological systems. J. Lipid. Res. 1998;39:1529-1542. DOI:https://doi.org/10.1016/S0022-

2275(20)32182-9
6. Hasiuk OM, Samoilenko YuS, Polovynko TO, Leonenko SIu. Fizychna pratsezdatnist v umovakh vplyvu erytropoez-stymuliuiuchoho faktoru. Pryrodnychyi almanakh (biolohichni nauky). 2017;23:5-12. http://na.kspu.edu/index.php/na/article/view/621.
7. Henning B, Chow CK. Lipid peroxidation and endothelial cell in-jury: Implications in atherosclerosis. Free Radic. Biol. Med. 1988;4:9–100. https://doi.org/10.1016/0891-5849(88)90070-6
8. Kadaja L, Eimre M, Paju K, Roosimaa M, Põdramägi T, Kaasik P, Pehme A, Orlova E, Mudist M, Peet N, Piirsoo A, Seene T, Gellerich FN, Seppet EK. Impaired oxidative phosphorylation in overtrained rat myocardium. Exp. Clin. cardiol. 2010;15(4):116-127.
9. Longini M, Belvisi E, Proietti F, Bazzini F, Buonocore G, Perrone S: Oxidative stress biomarkers: establishment of reference values for isoprostanes, AOPP, and NPBI in cord blood. Mediators Inflamm 2017:1758432, 2017. https://doi.org/10.1155/2017/1758432
10. Otterbrien LE, Bach FH, Alam J. et al. Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. Natur. Med. 2000;278(1):C92 - C101. https://doi.org/10.1038/74680
11. Petrache I, Otterbein LE, Alam J. et al. Heme oxygenase-1 inhibits (TNF)-ά – indused apoptosis in cultured fibroblasts. Am. J. Phisiol. Lung Cell. Mol. Physiol. 2000;278:L312-L329. https://doi.org/10.1152/ajplung.2000.278.2.L312
12. Vallance P, Collier J, Moncada S. Effects of endothelium-derived nitric oxide on peripheral arterior tone in man. Lancet.1989; 2: 997-1000. https://doi.org/10.1016/S0140-6736(89)91013-1
13. Vanoverschelde JL, Wijns W, Depre C. et al. Mechanisms of chronic regional postischemic dysfunction in humans. New insights from the study of noninfarcted collateral-dependent myocardium. Circulation. 1993;87:1513-1523. https://doi.org/10.1161/01.CIR.87.5.1513
14. Wang R, Alam G, Zagariya A, et al. Apoptosis of lung epithelial cells in response to TNF-alpha requires angiotensin II generation de novo. J. Cell. Physiol. 2000;185:253–259. https://doi.org/10.1002/1097-4652(200011)185:2<253::AID-JCP10>3.0.CO;2-%23
15. Yan HF, Tuo QZ, Yin QZ, Lei P. The pathological role of ferroptosis in ischemia/reperfusion-related injury. Zool Res. 2020;41(3):220-230. doi: 10.24272/j.issn.2095-8137.2020.042.
16. Кукоба Т. В., Мойбенко О.О. Гемоксигеназа та монооксид вуглецю: захист чи пошкодження клітин? Фізіол. Журнал. 2002;48(5):79-92.

REFERENCES

1. Abrahams S, Haylett WL, Johnson G, Carr JA, Bardien S. Antioxidant effects of curcumin in models of neurodegeneration, aging, oxidative and nitrosative stress: A review. Neuroscience. 2019;406:1-21. https://doi.org/10.1016/j.neuroscience.2019.02.020
2. Beschasnyi SP, Hasiuk OM. The carbon monoxide donor, topiramate, and blockers of aquaporine receptors decrease myocardial ischemia-reperfusion injiry. Fiziologichnyi Zhurnal. 2021;67(5):30-38. https://doi.org/10.15407/fz67.05.030
3. Bloomer RJ, Cole B, Fisher-Wellman KH. Racial differences in postprandial oxidative stress with and without acute exercise. Int. J. Sport Nutr. and Exercise Metabol. 2009;19 (5): 457-472. DOI: https://doi.org/10.1123/ijsnem.19.5.457
4. Bonekamp NA, Völkl A, Fahimi HD, Schrader M. Reactive oxygen species and peroxisomes: struggling for balance. Biofactors. 2009;35(4):346-355. https://doi.org/10.1002/biof.48
5. Girotti AW. Lipid hydroperoxide generation, turnover, and effectors action in biological systems. J. Lipid. Res. 1998;39:1529-1542. DOI:https://doi.org/10.1016/S0022-2275(20)32182-9

6. Hasiuk OM, Samoilenko YuS, Polovynko TO, Leonenko SIu. Fizychna pratsezdatnist v umovakh vplyvu erytropoez-stymuliuiuchoho faktoru. Pryrodnychyi almanakh (biolohichni nauky). 2017;23:5-12. http://na.kspu.edu/index.php/na/article/view/621.
7. Henning B, Chow CK. Lipid peroxidation and endothelial cell in-jury: Implications in atherosclerosis. Free Radic. Biol. Med. 1988;4:9–100. https://doi.org/10.1016/0891-5849(88)90070-6
8. Kadaja L, Eimre M, Paju K, Roosimaa M, Põdramägi T, Kaasik P, Pehme A, Orlova E, Mudist M, Peet N, Piirsoo A, Seene T, Gellerich FN, Seppet EK. Impaired oxidative phosphorylation in overtrained rat myocardium. Exp. Clin. cardiol. 2010;15(4):116-127.
9. Longini M, Belvisi E, Proietti F, Bazzini F, Buonocore G, Perrone S: Oxidative stress biomarkers: establishment of reference values for isoprostanes, AOPP, and NPBI in cord blood. Mediators Inflamm 2017:1758432, 2017. https://doi.org/10.1155/2017/1758432
10. Otterbrien LE, Bach FH, Alam J. et al. Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. Natur. Med. 2000;278(1):C92 - C101. https://doi.org/10.1038/74680
11. Petrache I, Otterbein LE, Alam J. et al. Heme oxygenase-1 inhibits (TNF)-ά – indused apoptosis in cultured fibroblasts. Am. J. Phisiol. Lung Cell. Mol. Physiol. 2000;278:L312-L329. https://doi.org/10.1152/ajplung.2000.278.2.L312
12. Vallance P, Collier J, Moncada S. Effects of endothelium-derived nitric oxide on peripheral arterior tone in man. Lancet.1989; 2: 997-1000. https://doi.org/10.1016/S0140-6736(89)91013-1
13. Vanoverschelde JL, Wijns W, Depre C. et al. Mechanisms of chronic regional postischemic dysfunction in humans. New insights from the study of noninfarcted collateral-dependent myocardium. Circulation. 1993;87:1513-1523. https://doi.org/10.1161/01.CIR.87.5.1513
14. Wang R, Alam G, Zagariya A, et al. Apoptosis of lung epithelial cells in response to TNF-alpha requires angiotensin II generation de novo. J. Cell. Physiol. 2000;185:253–259. https://doi.org/10.1002/1097-4652(200011)185:2<253::AID-JCP10>3.0.CO;2-%23
15. Yan HF, Tuo QZ, Yin QZ, Lei P. The pathological role of ferroptosis in ischemia/reperfusion-related injury. Zool Res. 2020;41(3):220-230. doi: 10.24272/j.issn.2095-8137.2020.042.
16. Kukoba T. V., Moibenko O.O. Hemoksyhenaza ta monooksyd vuhletsiu: zakhyst chy poshkodzhennia klityn? Fiziol. Zhurnal. 2002;48(5):79-92.

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