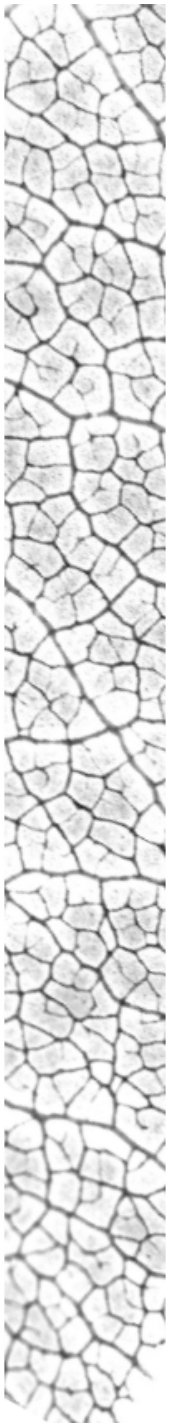

EurAsian Journal of BioSciences
Eurasia J Biosci 13, 957-963 (2019)

Carbamylated darbepoetin in combination with ethoxydol attenuates doxorubicin-induced cardiomyopathy in rats

Pavel D. Kolesnichenko [1*], Irina A. Popova [1], Dmitriy V. Sheblykin [1], Alhamzah Muhi Aldeen Azeez [1], Vladislav O. Soldatov [1], Sergey V. Povetkin [1], Galina A. Lazareva [1], Alexandr A. Stepchenko [1]

[1] Department of Pharmacology and Clinical Pharmacology, Medical Institute, Belgorod State University, Belgorod, RUSSIA
*Corresponding author: kolesnichenko_p@bsu.edu.ru

Abstract

Introduction: Doxorubicin is the drug of choice in the treatment of many malignant neoplasms, but its use is limited due to the risk of developing severe cardiomyopathy. This problem necessitates the prevention, early diagnosis and treatment of cardiomyopathy.

Material and Methods: The study was conducted on 80 male white Wistar rats, which were administered the following drugs during the experiment: doxorubicin (Teva) at a dose of 20 mg /kg, ethoxydol at a dose of 50 mg/kg, carbamylated darbepoetin at a dose of 50 mg/kg. The hearts of the rats were perfused in the installation of a Langendorf-isolated heart. All rats were recorded contractility indicators: maximum intraventricular pressure (IVP) (mm Hg), minimum IVP (mm Hg), average IVP (mm Hg), pulse IVP (mm. Hg), heart rate (HR, beats/min), maximum myocardial contraction rate (+ dP/dt_{max}, mm Hg/sec), maximum myocardial relaxation rate (-dP/dt_{max}, mm Hg/sec.), a test was performed with high-frequency stimulation. To assess the reserve capacity of the myocardium, we used the Tension-Time Index (tTTI) test of the "voltage over time" index.

Results: When used as a cardioprotector of ethoxydol at a dose of 50 mg/kg, a decrease in the toxic effect of DR and an improvement in performance by 14.8% compared with the group of DR. The degree of change in contractility indicators compared with the positive control group was 48.7%. As a result of CDEPO at a dose of 50 mg/kg, there is also a positive trend in the change in myocardial contractility, but to a lesser extent than in ethoxydol. The difference in performance compared with the DR group is 8.6%. The greatest cardioprotective effect was achieved by the introduction of a combination of DR and CDEPO. The increase in contractility compared with the doxorubicin group was 23.9%. The severity of changes in contractility indicators decreased to 38.5% compared with the positive control group. This trend in the dynamics of myocardial contractility indices can be traced both in the conditions of perfusion with norcalcium and hypercalcium solutions.

Conclusion: The most pronounced cardioprotective effect on the model of doxorubicin cardiomyopathy is determined by using a combination of carbamylated darbepoetin at a dose of 50 mkg/kg with ethoxydol at a dose of 50 mg/kg.

Keywords: Doxorubicin cardiomyopathy, carbamylated darbepoetin, ethoxydol, heart-isolated Langendorf

Kolesnichenko PD, Popova IA, Sheblykin DV, Aldeen Azeez AM, Soldatov VO, Povetkin SV, Lazareva GA, Stepchenko AA (2019) Carbamylated darbepoetin in combination with ethoxydol attenuates doxorubicin-induced cardiomyopathy in rats. Eurasia J Biosci 13: 957-963.

INTRODUCTION

The widespread demand for doxorubicin in medicine is due to the effectiveness and spectrum of its antitumor action. This cytostatic agent is the drug of choice for the treatment of most malignant tumors. However, the use of anthracycline drugs is limited due to the risk of developing severe cardiomyopathy, which is accompanied by progressive systolic dysfunction of the left ventricle (LV), leading to irreversible congestive heart failure (Danilenko 2018). This problem necessitates prevention, early diagnosis and treatment of cardiomyopathy. One of the current trends in the prevention of the occurrence of this side effect is the use of drugs with cardioprotective action. There are certain patterns in the interaction of drugs, manifested by potentiation, summation or additive effect, which greatly increases the chance of preventing the development of toxic side effects when using a combination of drugs with cardioprotective effects.

The search for substances capable of affecting the pathogenetic cascades of apoptosis and cell necrosis is

Received: February 2019
Accepted: June 2019
Printed: August 2019

one of the main tasks of modern pharmacology (Bogus et al. 2018, Denisyuk et al. 2016, Dzhimak et al. 2017, Gumanova et al. 2007, Kolesnichenko et al. 2018, Majorova et al. 2018, Peresypkina et al. 2019, Soldatov et al. 2018, Trauelsen et al. 2017). The main focus of research development is focused on oxidative stress (Dzhimak et al. 2017, Gumanova et al. 2007, Kolesnichenko et al. 2018, Peresypkina et al. 2019), inflammation (Denisuk et al. 2015, Denisyuk et al. 2016, Soldatov et al. 2018), nitrogen oxide imbalance (Korokina et al. 2018, Pokrovskii et al. 2017, Rajkumar et al. 2016) and intracellular secondary messengers (Gureev et al. 2014, Ragulina et al. 2017). Currently, one of the most effective drugs with tissue-protective properties is carbamylated darbepoetin, a modification of the glycoprotein hormone erythropoietin (EPO), also known as the hematopoietic hormone, but with a wide range of effects besides stimulating hematopoiesis (Gureev et al. 2014, Shabelnikova 2016, Shabelnikova et al. 2014, 2015) The ability to activate the phosphorylation of protein kinases, such as STAT-5, PI3K, Akt, Ras, the set of which varies according to the type of tissue (Kolesnichenko et al. 2018).

Also today, the use of antioxidants as a cardioprotector is important. A special role among substances based on natural metabolites is given to fumaric acid preparations. One of the promising ways to correct the level of metabolic acidosis and prolongation of energy production is the use of Krebs cycle intermediates (Danilenko et al. 2016, Kukes and Goroshko 2013). More attention is focused on easily assimilable intermediates - succinate and malate. The use of succinate is based on the fact that its oxidation reaction to fumarate occurs due to FAD-dependent dehydrogenase (the flavin nature of succinate dehydrogenase) and is independent of the presence of oxidized forms of NADH. In turn, another intermediate, malate, occupies a key position in the malate oxaloacetate cycle. Malate is the only substrate of the Krebs cycle, capable of reducing cytochrome b5 in the presence of NADH in low concentrations. Penetrating into the mitochondria, malate drops electrons on the respiratory chain and turns into oxaloacetate (ASC), which is again transformed into malate. The special role of malate in the regulation of redox processes in the myocardium is mediated by its ability to increase the respiratory control coefficient of mitochondria, restore cytochrome b5 in the presence of NADH, as well as a high content of malate dehydrogenase compared to other dehydrogenases of Krebs cycle substrates, and it also has an inhibitory effect common to antioxidants free radical processes (Kolesnichenko et al. 2018).

MATERIALS AND METHODS

The study was conducted on 80 male Wistar white rats. The study was conducted according to the method "Method for assessing the cardioprotective activity of pharmacological agents" (Danilenko et al. 2018). All rats were randomly divided into 5 groups. The first group - intact animals (positive control), intragastrically administered 0.9% NaCl. The second group, the control (negative control), doxorubicin (Teva) was administered intragastrically to the animals in a cumulative dose of 20 mg/kg once. The third group consisted of animals, which, against the background of a single intragastric administration of doxorubicin, were administered ethoxydol at a dose of 50 mg/kg. The fourth group includes animals injected with doxorubicin 20 mg/kg and carbamylated darbepoetin at a dose of 50 mg /kg. The fifth group - doxorubicin 20 mg/kg was administered against doxorubicin 20 mg/kg, ethoxydol 50 mg/kg and carbamylated darbepoetin 50 mkg/kg were injected against its background. After 48 hours, the hearts were removed from animals under combined anesthesia (Xyla premedication 0.5 ml/kg, basic anesthesia - Zoletil (30 mg/kg)), the aorta was cannulated and immediately connected to a heart-insulated Langendorf perfusion unit produced by Cardioprotect LLC, St. Petersburg (Russia).

In the perfusion column was a standard Krebs-Henseleite solution of the following composition (mmol): NaCl - 118.5; KCl - 4.7; $MgSO_4/7H_2O$ - 1.2; KH_2PO_4 - 1.2; $CaCl_2$ - 1.5; glucose - 11.1; $NaHCO_3$ –25.0. During the whole experiment, the pH and temperature of the solution were monitored by a pH-meter pH-410 and were 7.4±0.2 at 37 ° C. The oxygenation of the Krebs-Henseleite solution was carried out with carbogen (95% O_2, 5% CO_2). The pressure of the perfusate at the exit of the aortic cannula was 85±5 mm. Hg, the perfusate delivery rate exceeded the speed of passage through the system of coronary arteries by 3-5 times.

The contractile function of the heart was recorded using the "PhysExp Black Box" software and hardware complex manufactured by Cardioprotect LLC, St. Petersburg (the left ventricular diastolic pressure was 2–7 mm Hg), with which all rats conducted the registration of indicators of contractility: maximum intraventricular pressure (IVP) (mm Hg), minimum IVP (mm Hg), average IVP (mm Hg), pulse IVP (mm Hg), heart rate (HR, beats/min), the maximum rate of contraction of the myocardium ($+dP/dt_{max}$, mm Hg/sec), the maximum rate of myocardial relaxation ($-dP/dt_{max}$, mmHg/sec).

To determine the integral index reflecting the performance of calcium pumps of cardiomyocytes as a functional reserve of the heart, a hypercalcium solution was used under conditions of increasing frequency to 480 bpm. The calcium concentration in this solution was 5 mmol/l.

To create a high frequency (480 beats/min), electrical stimulator connectors were successively connected, the catecholamine mediators were washed, the heart was perfused for 20 minutes with a solution with a normal calcium content, then a hypercalcium

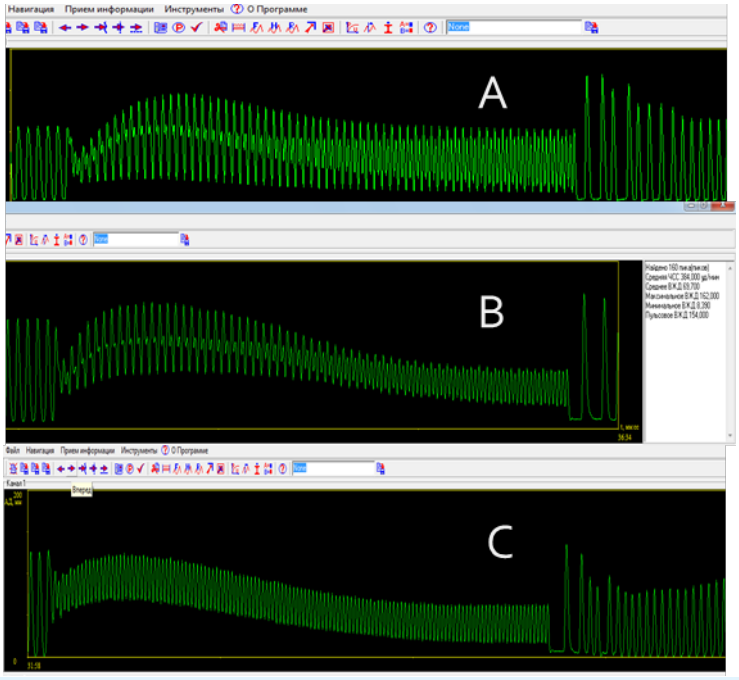

Fig. 1. Load test with submaximal electrostimulation of a Langendorff-isolated rat heart. A - positive control group (0.9% NaCl); B - negative control group (doxorubicin 20 mg/kg); C - the main group (doxorubicin 20 mg/kg + ethoxydol 50 mg/kg)

solution (5 mmol/L) for 5 minutes). After perfusion, the heart was stimulated with electrical pulses using the STM 200-1 instrument from BiopacSystems, Inc. (California, USA) for 15 seconds.

To assess the reserve capacity of the myocardium, we used the Tension-Time Index (tTTI) test of the "voltage over time" index calculated by the area under the curve of graphic recording of the minimum intraventricular pressure using the Origin pro 9.1 program. As an isoline, the value of the minimum IVP preceding stimulation was set.

Statistical data processing was carried out using the non-parametric Mann-Whitney test. The arithmetic mean and standard error of the mean were calculated.

RESULTS

After a single dose of doxorubicin in a dose of 20 mg/kg, changes in the initial parameters of myocardial contractility were noted after 20 minutes of stabilization with perfusion with a standard Krebs-Henselyayt solution, namely, a significant decrease in UR, + dp/dt and –dp/dt, which suggests cardiotoxic doxorubicin at a dose of 20 mg/kg (**Fig. 2** and **3**).

When using ethoxydol (E) on the model of doxorubicin cardiomyopathy, myocardial contractility indexes increase, the heart rate increases as compared with the negative control group, which indicates the cardioprotective effect of this substance. Also, when using carbamylated darbepoetin (CDEPO), against the background of doxorubicin cardiomyopathy, there is a positive trend in myocardial contractility indicators, but to a lesser extent than with ethoxydol. When using a combination of CDEPO and E, a significant improvement in myocardial contractility parameters is observed, their values approaching the level of the positive control group, which indicates a significant cardioprotective effect (**Fig. 2** and **3**).

At the next stage of the study, the effect of ethoxydol, carbamylated darbepoetin, and their combination under hypercalcium (5 mmol/l) solution with high-frequency cardiac stimulation was evaluated, since doxorubicin cardiomyopathy is based on myocardial calcium overload. At this stage, a "defect of diastole" is also defined.

When perfusing hearts in the Langendorff apparatus with a hypercalcium solution (5 mmol/l), a regular increase in the contractility parameter was found both in the group of intact animals and in groups with the simulation of doxorubicin cardiomyopathy.

There is also a pattern in the greater cardioprotective effect when using ethoxydol as compared to CDEPO. The most pronounced effect is observed when using a combination of these substances (**Fig. 2** and **3**).

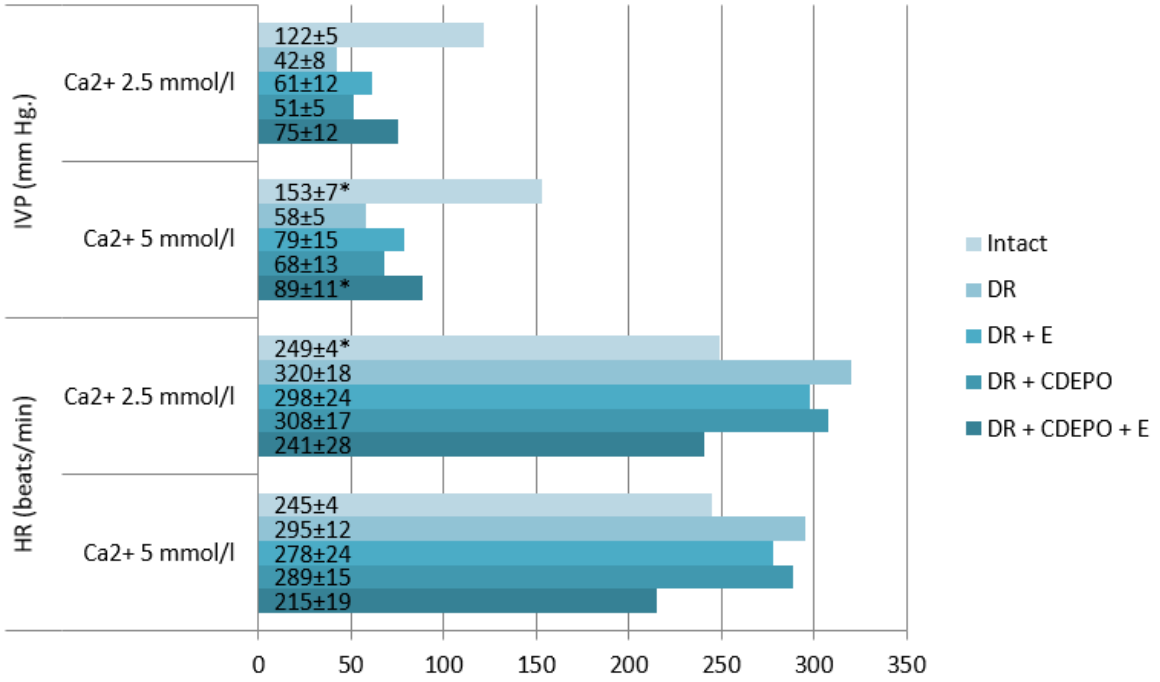

Fig. 2. Effect of ethoxydol, carbamylated darbepoetin and their combination on the parameters of myocardial contractility (HR and HR) of the rat hearts isolated according to Langendorf when simulating doxorubicin cardiomyopathy under conditions of perfusion with norm and hypercalcium solutions. (M±m; n=10)

Note: HR - heart rate (beats/min); IVP - intraventricular pressure (mm Hg.); * - p <0.05 relative to the group where doxorubicin was administered

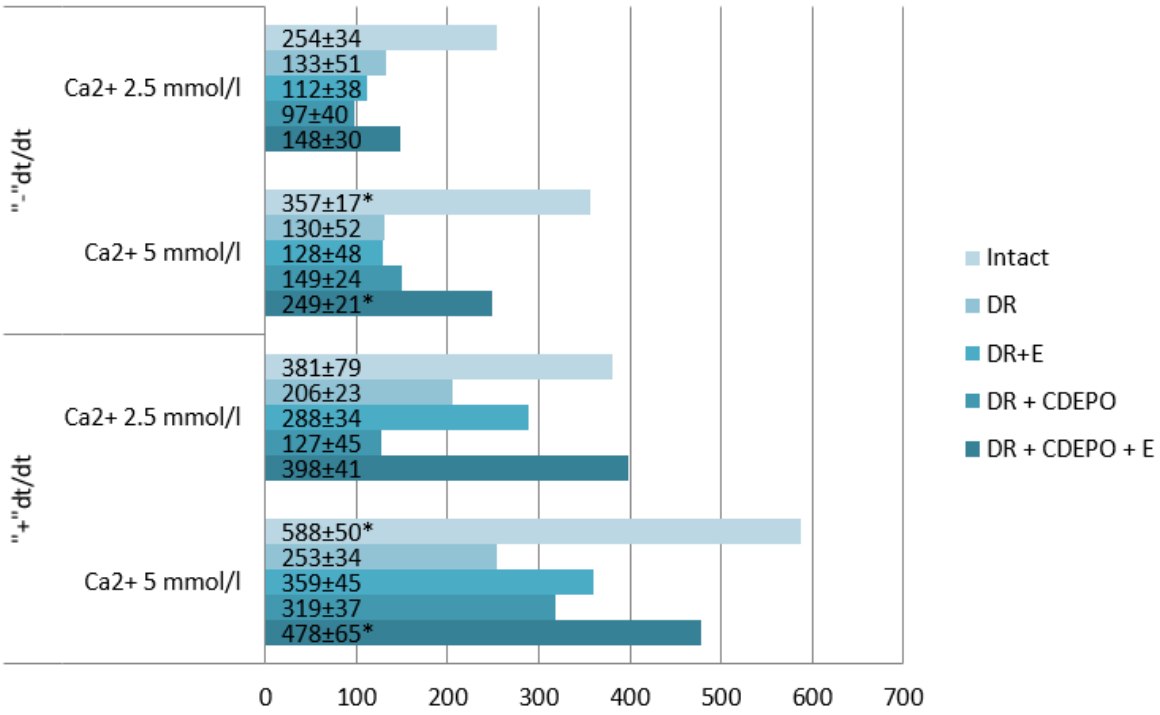

Fig. 3. Effect of ethoxydol, carbamylated darbepoetin, and their combination on the parameters of myocardial contractility (+ dt/dt, -dt/dt) isolated Langendorf hearts of rats when simulating doxorubicin cardiomyopathy under conditions of perfusion with normal and hypercalcium solutions. (M±m; n=10)

Note: + dp/dt; -dp/dt - the maximum rate of reduction/relaxation of the myocardium (mm Hg./s). * - p <0.05 relative to the group where doxorubicin was administered

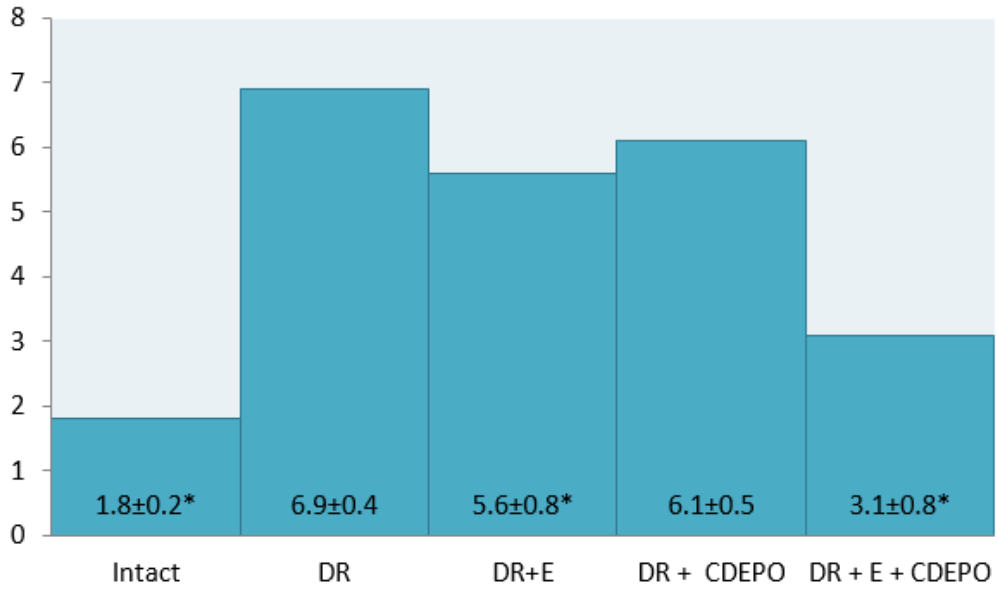

Fig. 4. Cardioprotective effect of ethoxidol, carbamylated darbepoetin, and their combination when performing a functional test with high-frequency cardiostimulation of hearts isolated by Langendorf after modeling doxorubicin cardiomyopathy
Note: StTTI - area under the intraventricular pressure curve for 15 seconds of the test for high-frequency cardiac stimulation in conditions of hypercalcium perfusion (standard units) * - p <0.05 relative to the group of doxorubicin

According to the test results with high-frequency electrostimulation and determination of Tension-Time Index (tTTI) by area along the curve of the minimum blood pressure in the simulation of doxorubicin cardiomyopathy, the area under the HR curve increased by 4 times, while using a combination of doxorubicin and CDEPO, the area is reduced by 41 % compared with the doxorubicin group (**Fig. 4**).

CONCLUSION

With the introduction of doxorubicin in a dose of 20 mg/kg, a significant decrease in the parameters of myocardial contractility, an increase in heart rate, is observed intragastrically. The severity of changes in indicators was 63.5% compared with the positive control group. When used as a cardioprotector of ethoxydol at a dose of 50 mg/kg, a decrease in the toxic effect of DR and an improvement in performance by 14.8% compared with the group of DR. The degree of change in contractility indicators compared with the positive control group was 48.7%. As a result of CDEPO at a dose of 50 mg/kg, there is also a positive trend in the change in myocardial contractility, but to a lesser extent than in ethoxydol. The difference in performance compared with the DR group is 8.6%. The greatest cardioprotective effect was achieved by the introduction of a combination of DR and CDEPO. The increase in contractility compared with the doxorubicin group was 23.9%. The severity of changes in contractility indicators decreased to 38.5% compared with the positive control group. This trend in the dynamics of myocardial contractility indices can be traced both in the conditions of perfusion with norcalcium and hypercalcium solutions.

When determining the area under the curve of the minimum HRV with the introduction of doxorubicin, its increase by 4 times is observed, which indicates the development of a “diastole defect”. The most pronounced decrease in the area under the curve induced the introduction of a combination of doxorubicin and CDEPO.

Since the greatest cardioprotective effect was revealed when using carbimylated darbepoetin and ethoxydol, we can speak of an additive type of interaction between these drugs.

REFERENCES

Bogus SK, Galenko-Yaroshevsky PA, Suzdalev KF, Sukoyan GV, Abushkevich VG (2018) 2-phenyl-1-(3-pyrrolidin-1-il-propyl)-1 H-indole hydrochloride (SS-68): Antiarrhythmic and cardioprotective activity and its molecular mechanisms of action (Part I). Research Results in Pharmacology, 4(2): 133-150. https://doi.org/10.3897/rrpharmacology.4.28592

Danilenko L, Klochkova G, Kizilova I, Pokrovskii M (2016) Metabolic cardioprotection: new concepts in implementation of cardioprotective effects of meldonium. Research Results in Pharmacology, 2(3): 95-100. https://doi.org/10.18413/2500-235X-2016-2-3-95-100

Danilenko LM (2018) Doxorubicin-associated Cardiomyopathy: New Approaches to Pharmacological Correction Using 3-(2,2,2-trimethylhydrazinium) Propionate Derivatives. Research Results in Pharmacology, 4(1): 81-86. https://doi.org/10.3897/rrpharmacology.4.25530

Danilenko LM, Timokhina AS, Khavanski AV, Pokrovskiy MV, Dovgan AP, Kolesnichenko, PD, et al. (2018) Method of analysis cardioprotective activity of pharmacological agents. Certificate of state registration of the PC, reg. № 2643104 from 30.01.2018. Rospatent

Denisuk TA, Pokrovskii MV, Philippova OV, Dolzhikov AA, Pokrovskaia TG, Korokin MV, et al. (2015) Endothelio- and cardioprotective effects of HMG-CoA reductase inhibitors under the condition of endotoxin-induced endothelial dysfunction. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 6(5): 1542-1547.

Denisyuk T, Lazareva G, Provotorov V, Pokrovskaya T (2016) Endothelium and cardioprotective effects of HMG-Co-A-reductase in combination with l-arginine in endothelial dysfunction modeling. Research Results in Pharmacology, 2(1): 4-8. https://doi.org/10.18413/2313-8971-2016-2-1-4-8

Dzhimak SS, Basov AA, Volchenko NN, Samkov AA, Fedulova LV, Baryshev MG (2017) Changes in the functional activity of mitochondria isolated from the liver of rat that passed the preadaptation to ultra-low deuterium concentration. Doklady Biochemistry and Biophysics, 476: 323–5. https://doi.org/10.1134/S1607672917050088

Gumanova NG, Artyushkova EB, Metel'skaya VA, Kochkarov VI, Pokrovskaya TG, Danilenko LM, et al. (2007) Effect of antioxidants pQ510 and resveratrol on regulatory function of the endothelium in rats with modeled arterial hypertension. Bulletin of Experimental Biology and Medicine, 143(6), 678-681. https://doi.org/10.1007/s10517-007-0212-x

Gureev VV, Alehin SA, Pokrovskiy MV, Dolghikov AA, Korokin MV, Gudyrev OS, Kolesnik IM (2014) Remote ischemic preconditioning correction in ADMA-like gestosis model. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 5(5): 1095-1098.

Kolesnichenko PD, Gudyrev OS, Zhuchenko MA, Zhernakova NI, Faitelson AV, Pershina MA, Soldatov VO (2018) Evaluation of carbamylated darbepoetin acute toxicity. International Journal of Research in Pharmaceutical Sciences, 9 (3): 725-730.

Kolesnichenko PD, Pershina MA, Gorbunova NS, Soldatov VO, Reznikov KM, Xenofontov AO (2018) The value of the redox potential of liquids in the dissolution of drugs in them. Russian Journal of Biopharmaceuticals, 10(4): 46-50.

Kolesnichenko PD, Reznikov KM, Zhhernakova NI, Stepchenko AA, Popova IA (2018) The Value Changes Redox System the Body Fluid Media for Life Processes and the Action of Drugs. Journal of International Pharmaceutical Research, 45: 440-444.

Korokina LV, Zhernakova NI, Korokin MV, Pokopejko ON (2018) Principles of pharmacological correction of pulmonary arterial hypertension. Research Results in Pharmacology, 4(2): 59-76. https://doi.org/10.3897/rrpharmacology.4.27732

Kukes VG, Goroshko VA (2013) Analysis of the effectiveness of the antioxidant action of the medicinal tools etilmetilgidroksipiridina malate (ethoxydole). Medicines and Rational Pharmacotherapy, 4: 26-28. (In Russian English abstract)

Majorova AV, Sysuev BB, Sokolova-Merkur'eva AV, Evseeva SB, Nesterova NI, Veselova VA, Puchenkova OA, Soldatov VO (2018) Gel with ectoine improves wound healing on a thermal burn model in rats. Journal of International Pharmaceutical Research, 45: 454-462.

Peresypkina A, Pazhinsky A, Pokrovskii M, Beskhmelnitsyna E, Pobeda A, Korokin M (2019) Correction of experimental retinal ischemia by l-isomer of ethylmethylhydroxypyridine malate. Antioxidants, 8(2). https://doi.org/10.3390/antiox8020034

Pokrovskii MV, Korokin MV, Kudryavtsev KV, Pokrovskaya TG, Gudyrev OS, Gureev VV, et al. (2017) Study of endothelial protective activity of phenol-derived thrombin and arginase-2 inhibitors KUD-259 and KUD-974. Bulletin of Experimental Biology and Medicine, 163(4): 436-438. https://doi.org/10.1007/s10517-017-3822-y

Ragulina V, Kostina D, Dovgan A, Burda Y, Nadezhdin S (2017) Nuclear factor kappa b as a potential target for pharmacological correction endothelium-associated pathology. Research Results in Pharmacology, 3(1): 114-124. https://doi.org/10.18413/2500-235X-2017-3-1-114-124

Rajkumar DSR, Densingh S, Gudyrev O, Faitelson A, Stepchenko A, Dolzhikov A, Povetkin S (2016) Study of the influence of L-norvaline, rosuvastatin and their combination on the level of microcirculation in bone tissue in experimental osteoporosis and fractures on its background. Research Results in Pharmacology, 2(1): 20-24. https://doi.org/10.18413/2313-8971-2016-2-1-20-24

Shabelnikova A (2016) Correction of ischemic damage to the retina on application of pharmacological preconditioning of recombinant erythropoietin. Research Results in Pharmacology, 2(2): 67-90. https://doi.org/10.18413/2313-8971-2016-2-2-67-90

Shabelnikova AS, Lutsenko VD, Pokrovskii MV, Peresipkina AA, Korokin MV, Gudyrev OS, et al. (2015) Protective effects of recombinant erythropoietin in ischemia of the retina: The role of mechanisms of preconditioning. Research Journal of Medical Sciences, 9(4): 200-203. https://doi.org/10.3923/rjmsci.2015.200.203

Shabelnikova AS, Peresypkina AA, Pokrovskiy MV, Kashuba AS, Netrebenko AS (2014) Analysis of the protective properties of erythropoetin and nicorandil on the basis of the model of the retina ischemia/reperfusion. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 5(6): 1335-1339.

Soldatov VO, Shmykova EA, Pershina MA, Ksenofontov AO, Zamitsky YM, Kulikov AL, Peresypkina AA, Dovgan AP, Belousova YV (2018) Imidazoline receptors agonists: possible mechanisms of endothelioprotection. Research Results in Pharmacology, 4(2): 11-18. https://doi.org/10.3897/rrpharmacology.4.27221

Trauelsen M, Rexen Ulven E, Hjorth SA, Brvar M, Monaco C, Frimurer TM, et al. (2017) Receptor structure-based discovery of non-metabolite agonists for the succinate receptor GPR91. Molecular Genetics and Metabolism, 6(12): 1585-1596. https://doi.org/10.1016/j.molmet.2017.09.005