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Comparative Analysis of the Effect of Beta Blockers of Different Generations on the Parameters of Myocardial Energy Metabolism in Experimental Doxorubicin-Induced Chronic Heart Failure

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Abstract: Beta blockers of three generations are first-line drugs in the treatment of chronic heart failure (CHF). However, to date there is no clear understanding of the effect of beta blockers on myocardial energy metabolism disorders in CHF. The aim of the study is to conduct a study of beta-blockers of different generations on myocardial energy metabolism in the experimental CHF. CHF was modeled in white outbred rats by administering doxorubicin. The study drugs were administered intragastrically – new drug Hypertril (1-(β-phenylethyl)-4-amino-1,2,4-triazolium bromide)-3.5 mg/kg, metoprolol - 15 mg/kg, Nebivolol -10 mg/kg, Carvedilol 50 mg/kg, Bisoprolol, 10 mg/kg. In the myocardium, the main indices of energy metabolism were determined - ATP, ADP, AMP, malate, lactate, pyruvate, succinate dehydrogenase (SDH) activity, NAD-dependent malate dehydrogenase (NAD-MDH) activity. Traditional second-generation beta-blockers (Metoprolol and Bisoprolol) did not affect the studied indices of energy metabolism, and third-generation beta-blockers with additional properties - Carvedilol and, especially, Nebivalol and Hypertril improved myocardial energy metabolism. Obtained results will help to expand our understanding of the effect of beta-blockers of various generations used to treat cardiovascular diseases on energy metabolism, and are also an experimental justification for the practical choice of these drugs in the complex therapy of CHF.

Keywords: chronic heart failure; beta blockers; energy metabolism; mitochondria; Hypertril

1. Introduction

It is known that in recent years the main cause of mortality and disability in the population of industrially developed countries is cardiovascular diseases [1,2]. Among all cardiovascular diseases, chronic heart failure (CHF) is a complex and major health problem in many countries. Despite the progress achieved over the past 20 years in the treatment of CHF, the problem remains relevant [3–5]. The reason for the importance of the problem of CHF is that this disease has an extremely unfavorable prognosis. Thus, the annual mortality rate among patients with functional class III-IV CHF reaches 60% and only half of less severe patients survive for 5 years from the date of diagnosis [6–9]. To date, recommendations have been developed for the treatment of CHF, which include the

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prescription of ACE inhibitors, diuretics, cardiac glycosides and beta blockers [10,11]. It is beta blockers, along with ACE inhibitors, that are considered first-line drugs in the treatment of CHF, as they can improve survival rates and hospitalization of patients, effectively increase ejection fraction (EF), reduce the mass and sphericity of the left ventricle (LV) [12]. The leading component of this effect of beta blockers is their cardioprotective properties [13]. Currently, depending on the pharmacological action, there are three generations of beta blockers.

First generation beta blockers - non-selective block β1- and β2-receptors (Propranolol (Anaprilin), Nadolol, Timolol), exhibit negative effects on carbohydrate and lipid metabolism, the central nervous system, erectile function in men, increase the tone of bronchial smooth muscles, vascular walls, myometrium, which significantly limits their use in clinical practice; Second-generation β-blockers are cardioselective, since they block only β1-receptors (Metoprolol, Bisoprolol, Atenolol), which have fewer side effects compared to first-generation drugs and have more favorable tolerability with long-term use and a convincing evidence base for long-term prognosis life during the treatment of CHF. Third generation β-blockers, which can be either highly selective for β1 receptors (Nebivalol, Betaxolol) or non-selective (Carvedilol, Labetalol, Carteolol), and have antiischemic, endotheliotropic, antioxidant, antiproliferative, antihypertrophic and antiapoptotic activity, with minimal side effects. Also, 1st and 2nd generation beta blockers are called traditional, and 3rd generation - beta blockers with additional pharmacological properties [14–16].

A potential drug Hypertril (1-(b-phenylethyl)-4-amino-1,2,4-triazolium bromide) exhibiting β1-blocking, vasodilatory, antihypertensive and cardioprotective effects and belonging to class IV toxicity (LD50 is 683.4 mg/kg after intragastric administration to rats) is a result of our multi-year efforts [17]. SPA "Pharmatron" together with the scientific and technological complex "Institute of Single Crystals" of the National Academy of Sciences of Ukraine has developed methods for the synthesis and standardization of the substance "Hypertril" and the technology of parenteral solutions (certificate No. 2, series 020213). According to the decision of the State Expert Center of the Ministry of Health of Ukraine, phase 1 has been successfully completed and phase 2 of clinical trials of Hypertril as an antihypertensive drug is underway. There are the first experimental positive results of using Hypertril for CHF and its advantages over other beta blockers [18,19].

It is known that CHF is accompanied by mitochondrial dysfunction and persistent disturbances in myocardial energy metabolism leading to macroerg deficiency, ROS production, oxidative stress and apoptosis [20,21]. Such disorders, on the one hand, can be considered as a component of the pathogenesis of CHF, and, on the other, as a key factor in its progression. The mechanism of action of traditional beta blockers and, especially, beta blockers with additional properties suggests the possibility of the drugs influencing myocardial energy metabolism when prescribed for CHF. However, these data are very limited and demonstrate a variable effect depending on the class of the drug. Thus, Propronalol (Anaprilin), a non-selective beta-blocker, enhances disturbances in myocardial energy metabolism due to a negative effect on mitochondria (inhibition of complex II of the respiratory chain, collapse of mitochondrial membrane potential) [22,23]. At the same time, Timolol had a mitoprotective effect and improved metabolism, directly related to the activity of myocardial mitochondria [24]. Interesting data have been obtained on the effect of a beta blocker with additional properties - Carvedilol - on energy metabolism [25].

Based on the above, we can outline the main direction of the ongoing research, which, in our opinion, will allow us to form a theoretical foundation for a clearer understanding of the possible and potential mechanisms of action of beta blockers of various generations on energy metabolism, which will, perhaps, resolve some practical issues, associated with the treatment of CHF. Purpose of the study: to investigate the effect of beta blockers of various generations (Bisoprolol, Metoprolol, Carvedilol, Nebivalol and the new drug Hypertril on indicators of myocardial energy metabolism in a model of doxorubicin-induced CHF.

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2. Materials and Methods

3.1. Animals

The experiments were carried out on 130 white outbred rats weighing 190-220g, obtained from the vivarium of the Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine and the Institute of Physiology. A.A. Bogomolets of the Academy of Medical Sciences of Ukraine. The duration of the quarantine (acclimatization period) for all animals was 14 days. During quarantine, each animal was examined daily (behavior and general condition), animals were observed twice a day in cages (morbidity and mortality). Before the start of the study, animals that met the inclusion criteria in the experiment were divided into groups using the randomization method. Throughout the experiment, the animals were observed, death was recorded, and their appearance was described. All manipulations were carried out in accordance with the provisions on the collection of animals for biomedical experiments (Strasbourg, 1986, as amended in 1998) and the "European Applicable Protection of Vertebrate Animals used for Experimental and Scientific Purposes". The protocols of experimental studies and their results were approved by the decision of the Commission on Bioethics of ZSMU (Protocol No. 3 dated March 22, 2021).

2.2. Experimental Model

Doxorubicin model was used to reproduce chronic heart failure [26]. The doxorubicin pharmacological model of CHF couldbe considered as the most effective, leading to the development of severe and progressive CHF in most animals. The use of doxorubicin (intraperitoneally at a cumulative dose of 15 mg/kg, divided into 6 injections for 14 days) leads to a decrease in left myocardial contractility, its eccentric remodeling, and the formation of progressive CHF in rats.

2.3. Drugs and Pharmacological Agents

The study used Doxorubicin "Ebeve" 50 mg/25 ml (EBEWE Pharma Ges.mbH Nfg. KG, Austria. All preparations were administered intragastrically once a day in the form of a suspension of 1% starch mucus for 30 days after a 14-day administration of doxorubicin - Hypertril at an experimentally substantiated dose of 3.5 mg/kg [17]. Metoprolol succinate - 15 mg/kg [26,27], Nebivalol 10 mg/kg [28], Carvedilol 50 mg/kg [29], Bisoprolol 10 mg/kg [30]. There were 10 animals in the intact group, 20 animals in the control and experimental groups. The following substances were used in the work: Hypertril substance (Scientific and technological complex "Institute of Single Crystals" of the National Academy of Sciences of Ukraine, certificate №2, series 020213), Metoprolol succinate tablets (Astra Zeneca UK Ltd, Sweden), Nebivalol tablets (Teva Pharmaceutical Industries, Ltd, Israel), Carvedolol tablets (Salutas Pharma GmbH, Germany), Bisoprolol tablets (Teva Pharmaceutical Industries, Ltd, Israel). Hypertril belongs to the class IV of toxicity (LD50 is 683.4 mg/kg with intragastric administration to rats). According to the decision of the State Expert Center of the Ministry of Health of Ukraine, Phase 1 of clinical trials of Hypertril was permitted, and successfully completed. Hypertril is currently undergoing Phase 2 of the clinical trials as an antihypertensive and antianginal drug.

2.4. Anaesthesia

Under the administration of thiopental anesthesia (40 mg/kg), rats from all experimental groups were taken out of the study. Following this, blood samples were obtained from the celiac artery for subsequent analysis.

2.5. Preparation of Biological Material Experimental Model

The heart was rinsed with chilled 0.15 M KCl (+4 0C) in a 1:10 ratio. After removing excess fat, connective tissue, and cutting out blood vessels and clots from internal cavities, the heart was washed again with 0.15 M KCl (+4 0C) in a 1:10 ratio. Subsequently, it was pulverized in liquid nitrogen to achieve a powdery consistency. On a WT500 torsion balance (manufactured in Moscow, Russia), 100 mg of heart tissue, previously ground into a fine powder using liquid nitrogen, was accurately

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weighed. The powdered tissue was then thoroughly mixed with 10.0 mL of a medium maintained at +20C. This medium contained the following components in millimoles per liter (mmol/L): sucrose (250 mmol/L), Tris-HCl buffer (20 mmol/L), and EDTA (1 mmol/L), adjusted to a pH of 7.4. The homogenate was subsequently subjected to a pre-centrifugation step using a Sigma 3-30k refrigerated centrifuge (Osterode am Harz, Germany) at (+4 0C) for 7 min at 1000 g to remove large cell fragments. The resulting supernatant was carefully collected and then subjected to a second centrifugation step at (+4 0C) for 20 min at 17,000 x g using the same Sigma 3-30k refrigerated centrifuge (Germany). The supernatant obtained from this step was collected and stored at -800C. The dense mitochondrial precipitate obtained after resuspension was utilized for further investigations.

2.6. Biochemical Assays and Techniques

The state of myocardial energy metabolism was judged by the content of ATP, ADP, AMP, malate, pyruvate, and lactate. And also by the activity of succinate dehydrogenase, malate dehydrogenase, the rate of opening of the mitochondrial pore and the charge of the mitochondrial membrane.

2.6.1. Determination of Adenyl Nucleotides by Thin Layer Chromatography

Principle of the method. The method is based on the separation of ATP, ADP and AMP in the dioxane-isopropanol-water-ammonia system on a thin layer of sorbent, followed by quantitative determination by direct spectrophotometry at 260 nm

Reagents:

- isopropanol;
- dioxane;
- ammonia;
- plates for thin layer chromatography on an aluminum substrate coated with a working layer of microfractionated silica gel sorbent grade.

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0.2 ml of protein-free tissue extract is applied to the starting line of the plate and chromatographed in the dioxane-isopropanol-water-ammonia system (4:2:4:1). ATP, ADP, AMP are identified in ultraviolet in the chromatolayer UVC - 365 nm. Samples are eluted in 4.0 ml of 0.1 N HCl and spectrophotometered at 260 nm. The content of ATP, ADP and AMP (μmol/g tissue) is calculated from the calibration curve, recalculated per sample of tissue.

2.6.2. Quantitative Determination of Pyruvate Content Using the Zoch-Lamprecht Method

Principle of the method. In the presence of lactate dehydrogenase (LDH), pyruvate is reduced to lactate:

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \leftrightarrow \text{lactate} + \text{NAD}^+ \quad (1$$

The amount of pyruvate used in the reaction is equimolar to the amount of NADH, the decrease of which is determined at a wavelength of 340 nm.

Reagents:

– 0.5 M tris-HCl-aminomethane buffer, pH 7.6;

– 0.06 M NADH solution;

– lactate dehydrogenase (activity 700 units/mg).

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0.8 ml of protein-free extract is added to 1.2 ml of Tris-HCl buffer. The reaction is started by adding 0.05 ml of LDH solution. The optical density is measured before starting the reaction (E1) and after 4 minutes (E2).

The amount of pyruvate is calculated using the formula:

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$$C = \frac{\Delta E \cdot K \cdot V}{6{,}22}, \quad (2$$

where: ΔE (E2 – E1);
V - is the final sample volume in the cuvette;
K - is the dilution factor of the sample relative to the tissue.

2.6.3. Quantitative Determination of Malate Using the Hohorst Methods

Principle of the method. In the presence of malate dehydrogenase (MDH), malate is converted to oxaloacetic acid. Binding of oxaloacetic acid with hydrazine-glycine buffer ensures complete oxidation of malate.

$$\text{Malate} + NAD^+ + \text{hydrazine} \leftrightarrow \text{oxaloacetate-hydrazine} + NADH + H_2O \quad (3$$

The formation of the reduced form of NADH is equimolar to the amount of oxidized malate, the increase of which is recorded at 340 nm.

Reagents:
- 0.4 M hydrazine-glycine buffer, pH 9.5;
- 0.05 M NAD+ solution;
- malate dehydrogenase (activity 700 units/mg).

Conducting research.

0.2 ml of protein-free brain extract is placed in 2.2 ml of hydrazine-glycine buffer. The reaction is started by adding 0.2 ml of NAD+ solution. The optical density is measured before starting the reaction (E1) and after 4 minutes (E2).

The amount of malate is calculated using the formula:

$$C = \frac{\Delta E \cdot K \cdot V}{6{,}22}, \quad (4)$$

where: ΔE (E2 – E1);
V - is the final sample volume in the cuvette;
K - is the dilution factor of the sample relative to the tissue.

2.6.4. Determination of Succinate Dehydrogenase (SDH) Activity

Principle of the method.

Under the influence of SDH, succinic acid reduces potassium hexacyanoferroate (III) K3[Fe(CN)6], which has a yellow color, to colorless potassium hexacyanoferroate (II) K4[Fe(CN)6]. The activity of the enzyme is proportional to the amount of hexacyano-ferroate (III) reduced.

Reagents:
– 0.1 M succinic acid;
– 25 mM potassium hexacyanoferroate (III);
– 150 mM sodium azide;
– 25 mM EDTA (pH 7.8);
– 0.1 M phosphate buffer (pH 7.8);
– 20% trichloroacetic acid (TCA).

Conducting research.

1 ml of 0.1 M phosphate buffer and 0.1 ml of solutions of succinic acid, EDTA, sodium azide, and distilled water are poured into centrifuge tubes. After this, 0.5 ml of the test tissue is added to the reaction medium and incubated for 5 minutes to inhibit cytochrome oxidase with sodium azide. The reaction is started by adding 0.1 ml of potassium hexacyanoferroate(III) solution. Samples are incubated for 10-15 minutes at 30°C. After incubation, the reaction is stopped by immersing the samples in ice and adding 2 ml of 20% TCA. To control samples containing all components of the incubation mixture, TCA is added before adding the tissue homogenate. Thus, SDH in control

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samples is completely denatured from the beginning of incubation, and no specific reduction occurs. After stopping the reaction and cooling, the samples are centrifuged at 2000 rpm (at a temperature of 15°C) for 15 minutes to precipitate denatured protein. The supernatant is photometered at 420 nm. A mixture of 20% TCA and 0.1 M phosphate buffer (1:1) serves as an optical control. To determine the content of potassium hexacyanoferroate (III) in samples, a calibration curve is constructed based on the results of photometry of samples containing from 100 to 1000 μg of potassium hexacyanoferroate (III) in 4 ml of solution. The amount of potassium hexacyanoferroate (III) reduced during incubation is calculated from the difference in extinctions (Ek – Epr). Enzyme activity (nmol succinate/min per 1 mg protein) is calculated using the formula:

$$A = 1000 \cdot m / 2M \cdot a \cdot t, \quad (5)$$

where: m is the amount of reduced potassium hexacyanoferroate (III) in the sample;
a – protein content in the sample, mg;
M – molecular weight of potassium hexacyanoferroate (III);
t – incubation time, min.

2.6.5. Determination of lactate content using the Hohorst method

Principle of the method. In the presence of lactate dehydrogenase (LDH), lactate is converted to pyruvate, and the binding of pyruvate formed during the reaction with a hydrazine-glycine buffer promotes complete oxidation of lactate.

$$\text{Lactate} + NAD^+ + \text{hydrazine} \circledcirc \text{hydrazine-pyruvate} + NADH + H_2O \quad (6)$$

The formation of the reducing form of NAD is equimolar to the amount of oxidized lactate, the increase of which is recorded at 340 nm. 0.4 M hydrazine-glycine buffer pH 9.5;

- 0.05 M NAD solution;
- lactate dehydrogenase (activity 700 units/mg).

Conducting research.

0.2 mg of protein-free tissue extract is added to the incubation mixture consisting of 2.0 ml of hydrazine-glycine buffer and 0.2 ml of NAD solution. The optical density is measured before starting the reaction (E1) and after 4 minutes (E2).

The amount of lactate is calculated using the formula:

$$C = \frac{\Delta E \cdot K \cdot V}{6{,}22}, \quad (7)$$

2.6.6. Determination of NAD-Dependent Malate Dehydrogenase Activity

Principle of the method. The activity of NAD-dependent malate dehydrogenase was studied in the direct malate dehydrogenase reaction, in which the amount of oxidized malate is equimolar to the amount of reduced NAD. The increase in NADH concentration in the samples was recorded at 340 nm. Carrying out the reaction in an alkaline medium promotes a shift in the reaction towards the oxidation of malate.

Reagents: Incubation medium: Sodium glycine buffer – 85 mM (pH 10.0); D;L-sodium malate - 85mM; NAD-25mM

Progress of determination The reaction was started by adding 0.1 ml of tissue extract to 2.9 ml of incubation medium. The optical density was measured immediately (E1) and its increase after 3 min E2 (wavelength 340 nm). Enzyme activity was expressed in μmol of NADH formed in 1 min per 1 mg of protein (NAD/min per 1 mg of protein). Enzyme activity was calculated using the formula:

$$X = EV/6.22a, \quad (8$$

where: E - change in optical density at 340 nm in 3 minutes;
V - final sample volume (3 ml);
6.22 micromolar extinction coefficient of NADH at 340 nm;

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a is the protein concentration in the sample.

2.6.7. Opening of the Mitochondrial Pore (MP)

Determination procedure: A suspension of mitochondria (1 mg of protein per sample) is added to the incubation medium (70 mM sucrose, 5 mM HEPES, 70 mM KCl, 0.5 - 1 mM KH2PO4, pH 7.4). The opening of the MP is determined at λ = 540 nm at 25 0C with constant stirring for 25 minutes.

The membrane potential (MPM) (Ψ) of mitochondrial charge is measured in the presence of safronin-O. Determination progress: The potential generated on the inner mitochondrial membrane was recorded on a spectrophotometer, in dual-wave mode (511 – 533 nm), with safranin O as a voltage-dependent probe (18 μM). The measurements were carried out in a 10x10 mm glass cuvette with a working volume of 2 ml. The measurements were carried out in a sodium environment. The sodium medium contained: 0.62 mM NaCl; 40 mM Caps (3-[cyclohexylamino]-1-propanesulfonic acid)-NaOH (pH = 10). The protonophore uncoupler FCCP (p-trifluoromethoxyphenylhydrazone) and the antiporter monensin were used to dissipate the potential. The swelling of mitochondria was recorded on a spectrophotometer by a decrease in the optical density of the mitochondrial suspension at 540 nm.

To carry out biochemical studies, we used an Eppendorf BioSpectometr (USA) spectrophotometer and an Agilent Fluorescence-spectrophotometer (USA).

2.7. Statistical Analysis

Statistical analyses were conducted using "STATISTICA® for Windows" (StatSOFT, Hamburg, Germany). Group comparisons were assessed using either one-way ANOVA or ANOVA for repeated measurements, followed by post hoc Bonferroni correction or the Kruskal–Wallis criterion with subsequent Dunn correction. Statistical significance was determined at a threshold of $p < 0.05$.

3. Results and Discussion

Biochemical studies revealed disturbances in myocardial energy metabolism and energy deficiency in the group of animals with CHF. Thus, a 14-day administration of doxorubicin led on the 45th day of the experiment to a decrease in the level of ATP in the myocardial cytosol by 50% and in mitochondria by 55%. In parallel, a decrease in the ADP content in the myocardial cytosol of rats with CHF by 42% was recorded against the background of an increase in the ADP level by 75.3% (Table 1). Our results correspond to generally accepted ideas about disturbances in the energy supply of the myocardium under conditions of ischemia [31].

Table 1. The influence of Hypertril and the reference drug on the content of adenyl nucleotides in the cytosolic fraction of the myocardium of animals with experimental CHF.

Group of animals	ATP, μmol/g tissue	ADP, μmol/g tissue	AMP, μmol/g tissue
Intact (*n*=10)	3.8 ± 0.21	0.57 ± 0.02	0.154 ± 0.007
CHF (control) (*n*=6)	1.9 ± 0.12[1]	0.33 ± 0.017[1]	0.27 ± 0.014[1]
CHF+ Hypertril, 3.5 mg/kg (*n*=19)	2.7 ± 0.12*[1]	0.41 ± 0.010*[1]	0.169 ± 0.011*[1]
CHF+Carvedilol 50 mg/kg (*n*=10)	2.2 ± 0.09[1]	0.36 ± 0.017[1]	0.25 ± 0.017[1]
CHF+Nebivalol, 10 mg/kg (*n*=16)	2.3 ± 0.07*[1]	0.36 ± 0.021[1]	0.23 ± 0.011*[1]
CHF + Bisoprolol, 10 mg/kg (*n*=16)	1.85 ± 0.14[1]	0.35 ± 0.033[1]	0.26 ± 0.034[1]
CHF + Metoprolol, 15 mg/kg	1.9 ± 0.11[1]	0.34 ± 0.054[1]	0.25 ± 0.017[1]

In parentheses the number of animals that survived at the end of the experiment; * - changes are significant in relation to animals in the control group ($p<0.05$);; 1 - changes are significant in relation to animals of the intact group ($p<0.05$).

We also recorded a 70% malate deficiency in the cytosolic fraction with a 62% decrease in the activity of mitochondrial NAD-dependent MDH (Table 2 and Table 3), which indicates possible

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inhibition of the malate-aspartate shuttle mechanism of transport of reduced equivalents into the mitochondria [32,33] and the formation of secondary mitochondrial dysfunction.

Table 2. The effect of Hypertril and reference drug on the content of energy metabolism intermediates in the cytosol of the heart of animals with experimental CHF.

Group of animals	Lactate, μmol/g tissue	Malate, μmol/g tissue	Pyruvate, μmol/g tissue
Intact (*n*=10)	5.2 ± 0.31	0.36± 0.02	0.15± 0.01
CHF (control) (*n*=6)	12.1 ± 0.87[1]	0.11± 0.01[1]	0.071± 0.001[1]
CHF+ Hypertril, 3.5 mg/kg (*n*=19)	7.4 ± 0.44*[1]	0.21± 0.01*[1]	0.088± 0.001*[1]
CHF+Carvedilol 50 mg/kg (*n*=10)	10.2 ± 0.92[1]	0.12± 0.02	0.072± 0.001[1]
CHF+Nebivalol, 10 mg/kg (*n*=16)	8.10± 0.54*[1]	0.14± 0.01*[1]	0.080± 0.001*[1]
CHF + Bisoprolol, 10 mg/kg(*n*=16)	11.5 ± 0.42[1]	0.12± 0.01[1]	0.072± 0.003[1]
CHF + Metoprolol, 15 mg/kg	11.1 ± 1.2	0.12± 0.01[1]	0.065± 0.002

In parentheses the number of animals that survived at the end of the experiment; * - changes are significant in relation to animals in the control group ($p<0.05$);; 1 - changes are significant in relation to animals of the intact group ($p<0.05$)

Table 3. The effect of Hypertril and reference drug on energy metabolism indices in the mitochondrial fraction of cardiac tissue of animals with experimental CHF.

Group of animals	Lactate, μmol/g tissue	NAD-MDH, μmol/mg protein/min	SDH, nmol/mg protein/min	IP opening, Δ mitochondrial E 540 nm	membrane potential, (Ψ)	ATP, μmol/g tissue
Intact (*n*=10)	1.5 ±0.08	1.71± 0.08	5.5± 0.28	0.052±0.002	52.1±3.2	2.7 ± 0.12
CHF (control) (*n*=6)	2.7 ±0.14[1]	0.65± 0.04[1]	1.8± 0.10[1]	0.63± 0.022[1]	18.2±1.0[1]	1.2 ± 0.03[1]
CHF+ Hypertril, 3.5 mg/kg (*n*=19)	1.7 ±0.15*	1.14± 0.08*[1]	3.1±0.17*[1]	0.31±0.005*[1]	33.2±2.4*[1]	1.60 ± 0.15*[1]
CHF+Carvedilol 50 mg/kg (*n*=10)	2.5±0.19[1]	0.67± 0.01[1]	2.2± 0.14*[1]	0.44±0.011*[1]	25.4±1.2*[1]	1.3 ± 0.11[1]
CHF+Nebivalol, 10 mg/kg (*n*=16)	2.2 ±0.10*[1]	0.79± 0.04*[1]	2.6±0.11*[1]	0.47±0.007*[1]	25.7±1.8*[1]	1.42 ± 0.07*[1]
CHF + Bisoprolol, 10 mg/kg (*n*=16)	2.3±0.18*[1]	0.67± 0.02[1]	2.0± 0.22[1]	0.63±0.015[1]	18.8±2.7[1]	1.2 ± 0.25[1]
CHF + Metoprolol, 15 mg/kg	2.5±0.21[1]	0.68± 0.03[1]	2.1± 0.19[1]	0.65±0.011[1]	19.3±3.0[1]	1.2 ± 0.10[1]

In parentheses the number of animals that survived at the end of the experiment; * - changes are significant in relation to animals in the control group ($p<0.05$);; 1 - changes are significant in relation to animals of the intact group ($p<0.05$)

Among the causes of mitochondrial dysfunction in CHF are oxidative stress, disruption of NO biosynthesis, production of its cytotoxic derivatives, and development of nitrosating stress [34–36]. It is currently known that the main manifestations of mitochondrial dysfunction are a decrease in the level of ATP in the cell, an increase in the level of lactate and a decrease in pyruvate, activation of cell death mechanisms and the production of reactive oxygen species (ROS) by mitochondria [37]. Currently, the effect of impaired ATP synthesis in mitochondria on the functional activity of the myocardium has been studied to the greatest extent [38]. It has been established that with a decrease in the content of ATP in the mitochondria and cytosol of the myocardium by 10–20%, the activity of all energy-dependent processes decreases by 80%. The effects of an insufficient amount of ATP include suppression of the disruption of ion pumps, ion homeostasis and, accordingly, the contractile function of the heart [39]. Inhibition of energy production processes in the mitochondria of

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cardiomyocytes is accompanied by a weakening of lipid beta-oxidation, which results in a violation of lipid homeostasis in the cell and the accumulation of acyl-CoA thioesters, acylcarnitines, ceramides and triglycerides, which enhance the formation of myocardial hypertrophy in CHF [40,41].

Under the influence of mitochondria-formed ROS, there is an increase in the opening of mitochondrial pores, expression and release of proapoptotic proteins into the cytosol. The opening of the pores occurs due to the oxidation of the thiol groups of the cysteine-dependent region of the protein of the inner mitochondrial membrane (ATP/ADP antiporter) by cytotoxic derivatives of NO, which turns it into a permeable non-specific channel - a pore [42,43]. ROS generated by mitochondria also participate in the transmission of intracellular signals of receptors for endothelin, TGF-β1, PDGF, AT-II, FGF-2, etc. ROS are also capable of changing the activity of various transcription factors, including NF-κB, AP-1, and the proapoptotic protein p66Shc. In general, an increase in ROS production, by affecting the intracellular signaling mechanisms discussed above, can contribute to the activation of the inflammatory process in heart tissue, the development of hypertrophic and fibrotic changes [44,45].

A course of administration of Hypertril tablets to rats with CHF resulted in a reduction in manifestations of secondary mitochondrial dysfunction. Thus, in animals treated with a course of Hypertril, there was a decrease in the opening of the mitochondrial pore (MP) by 51% ($p < 0.05$), as well as an increase in the charge of the inner membrane of the myocardial mitochondria by 82% ($p < 0.05$) compared to the group of untreated animals (Table 3). In these indicators, Hypertril is superior to the action of both traditional beta-blockers - Metoprolol and Bisoprolol, as well as Carvedilol and Nebivalol. Biochemical studies of the myocardium of rats with CHF made it possible to identify the features of the mitoprotective and anti-ischemic action of Hypertril. Thus, the ATP content in the cytosolic and mitochondrial fractions in rats treated with Hypertril increased ($p < 0.05$) by 42% and 33%, respectively. Along with an increase of 24% in the ADP content and a decrease of AMP by 54% were observed in the cytosol of the heart ($p<0.05$) compared with the corresponding indicators of the control group (Tables 1-3). In the cytosolic and mitochondrial fractions of the myocardial homogenate of animals with myocardial infarction under the influence of Hypertril, a decrease in lactate by 38.8 and 37%, respectively, was observed, which indicated a decrease in the activity of low-productive glycolysis (Table 2). At the same time, in the myocardial mitochondria of rats with CHF treated with Hypertril, the activity of SDH increased by 72% ($p < 0.05$) and NAD-MDH by 75.3% ($p < 0.05$) compared with the group of untreated animals. In the cytosolic fraction of the myocardial homogenate of rats with CHF, against the background of Hypertril administration, the level of malate by 90% and pyruvate by 24% significantly increased. The positive changes in the myocardium of animals under the influence of Hypertril indicate a decrease in the manifestations of mitochondrial dysfunction and activation of compensatory cytosolic-mitochondrial shunts of ATP synthesis and a decrease in energy deficiency. Administration of Nebivalol to rats with CHF also had a positive effect on the energy metabolism of the myocardium. Thus, Nebivalol reduced mitochondrial swelling by 25% ($p < 0.05$) and increased the mitochondrial charge by 41% ($p < 0.05$) compared to the parameters of the group of untreated animals. Nebivalol increased the concentration of ATP in the cytosol and mitochondria of the myocardium of rats with CHF by 21% ($p < 0.05$) and 18% ($p < 0.05$), respectively, against the background of a decrease in AMP ($p < 0.05$) compared to the control. Administration of Nebivalol increased the activity of SDH by 44% ($p < 0.05$) and NAD-MDH by 21.5% ($p < 0.05$) in the myocardial mitochondria of rats with CHF compared to the control. Administration of Nebivalol resulted in a decrease in lactate (in the cytosol by 33% and in the mitochondria by 18%) ($p < 0.05$) and an increase in malate by 27% ($p < 0.05$) and pyruvate by 12% ($p < 0.05$) in the cardiac cytosol of rats with CHF compared to the group of untreated animals with CHF.

Administration of Carvedilol had a significant effect on the indices of mitochondrial swelling reduction (30%) and mitochondrial charge increase (39%) in the myocardium of rats with CHF (Table 3). Carvedilol also significantly increased the activity of SDH (15%) in the mitochondria of rats with CHF compared to the control. Administration of Carvedilol had no significant effect on the other studied indices of energy metabolism in the myocardium of rats with CHF (Tables 1-3). Traditional beta-blockers – Metoprolol and Bisoprolol had no significant effect on the studied indices of energy

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metabolism in the myocardium of rats with CHF (Tables 1-3). The only thing that attracts attention in the group of rats with CHF that received a course of Bisoprolol in the mitochondrial fraction of the myocardium was a decrease in lactate by 14% ($p<0.05$) compared to the control.

Analyzing the obtained results of biochemical studies of myocardial energy metabolism in experimental CHF and against the background of the use of Hypertril, it can be concluded that the starting mechanism of the anti-ischemic action of Hypertril is its effect on the dysfunction of the mitochondria of cardiomyocytes. Apparently, Hypertril, by reducing the damaging effect of ROS and free radicals on the SH-groups of the cysteine-dependent region of the protein of the inner mitochondrial membrane, prevents the opening of the mitochondrial pore and maintains the functional activity of the mitochondria, which subsequently improves the energy metabolism of the myocardium under ischemic conditions [42]. This statement is also confirmed by our previous study, which showed that Hypertril, unlike Metoprolol, Bisoprolol, Carvedilol and Nebivalol, leads to a decrease in systolic and diastolic dysfunction, restoration of autonomic mechanisms of heart rhythm regulation, a decrease in the amplitude of the ST interval ($p < 0.05$), which, in combination with the restoration of the amplitude of the R wave, indicates the preservation of high performance of cardiomyocytes in doxorubicin-induced CHF [19]. The mechanism of such an effect of Hypertril on energy metabolism parameters in rats with CHF is apparently associated not only with its β1-adrenergic blocking effect, but is also possibly realized through additional mechanisms identified earlier – antioxidant and NO-mimetic [46]. The viability of such assumptions is based on various studies that have shown that patients with mitochondrial disorders have a deficiency of NO, and the administration of NO-mimetics leads to an improvement in mitochondrial function and energy metabolism [47,48].

Carvedilol, a β-adrenergic receptor antagonist with strong antioxidant activity, provides a high degree of cardioprotection in various experimental models of ischemic heart injury. Data on the effect of Carvedilol on mitochondrial bioenergetic functions and ROS formation have been obtained. Thus, Carvedilol is able to reduce the formation of H_2O_2, increase the level of reduced glutathione, and restore mitochondrial respiration due to its antioxidant effect [49]. Carvedilol exhibits the properties of an ROS scavenger and also inhibits the formation of ROS in mitochondria due to "soft uncoupling" and a slight decrease in the potential of the mitochondrial membrane; it is able to directly protect the ultrastructure of mitochondria, reduce Ca++ overload of mitochondria, but does not affect the indicators of mitochondrial respiration after 7-week administration of doxorubicin [50,51]. It has been shown that the direct mitoprotective properties of Carvedilol are associated with its properties to suppress the formation of ROS in the xanthine oxidase reaction of mitochondria and by increasing the activity of cytosolic Cu, Zn-SOD and mitochondrial Mn-SOD, as well as catalase [51,52]. It has also been shown that "antioxidant" concentrations of Carvedilol and its metabolite BM-910228 do not affect mitochondrial respiration parameters [50]. Some studies have shown that Carvedilol, due to its uncoupling effect, can also exhibit prooxidant properties [25].

Our results, which show that Metoprolol does not have a reliable effect on the energy metabolism of the myocardium, coincide with the data of other researchers. Thus, it was shown that this selective beta-blocker, prescribed for the treatment of CHF [12], does not improve the mitochondrial ultrastructure after the introduction of doxorubicin, did not reduce peroxidation processes, did not reduce the degree of Ca^{++} overload of mitochondria [53–55]. Metoprolol attenuates post-infarction structural remodeling without concomitant improvement in myocardial energy metabolism in rats with chronic CHF [56]. Bisoprolol was the first beta blocker to show clinical efficacy in heart failure [57]. Our results demonstrated the absence of a clear reliable effect of Bisoprolol on myocardial energy metabolism in CHF (except for the effect on LDH), which is consistent with other researchers [58]. It has been shown that the protective effect of Bisoprolol on the heart is not associated with the optimization of energy metabolism, but has other mechanisms [59]. Bisoprolol has also been shown to inhibit mitochondrial respiration and ATP synthesis in cancer cells [60]. It has been shown that blockade of cardiac β1 receptors with traditional beta blockers via the PKA/cAMP signaling pathway suppresses the nuclear-encoded mitochondrial protein IF1 and inhibits oxidative phosphorylation in cardiac mitochondria [60]. Nebivalol is a latest generation beta

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blocker with additional metabolitotropic properties – NO-mimetic and antioxidant, and is actively used in the treatment of arterial hypertension and CHF [61]. To date, there are no complete data on the effect of Nebivalol on myocardial energy metabolism in CHF, and it is difficult for us to compare our modest results with the data of other researchers. It is known that Nebivalol is an ROS scavenger and is able to protect mitochondrial membranes, affect various mechanisms of mitoptosis, increase ATP, creatine phosphate and normalize the [lactate]/[pyruvate] ratio. Moreover, the effect on energy metabolizum is not associated with its NO-mimetic effect [62,63]. There are, however, other studies demonstrating the involvement of Nebivalol in enhancing the formation of mitochondrial dysfunction in cancer cells [60,64]. Other researchers do not confirm a direct negative effect of Nebivalol on mitochondria in non-tumor cells, which emphasizes its specificity and excludes any antimitotic toxicity [60,65].

4. Conclusions

Thus, the conducted studies have established an ambiguous effect of beta-blockers on myocardial energy metabolism in the doxorubicin model of CHF. Traditional second-generation beta-blockers (Metoprolol and Bisoprolol) did not affect the studied indices of energy metabolism, and third-generation beta-blockers with additional properties - Carvedilol and, especially, Nebivalol and Hypertril improved myocardial energy metabolism. The use of these drugs and, especially, Hypertril in CHF led to an increase in the content of ATP and ADP against the background of a decrease in AMP and lactate with a simultaneous increase in the activity of SDH, NAD-MDH and malate concentration in various fractions of the myocardial homogenate of rats with CHF. Beta-blockers with additional properties and, especially, Hypertril, reduced the opening of the mitochondrial pore (MP) and increased the charge of the inner membrane of the myocardial mitochondria. The revealed facts indicate a decrease in the manifestations of mitochondrial dysfunction and activation of compensatory cytosolic-mitochondrial shunts of ATP synthesis and a decrease in energy deficiency in the myocardium of rats with CHF when prescribing beta-blockers with additional properties and, especially Hypertril. The results obtained will help to expand our understanding of the effect of beta-blockers of various generations used to treat cardiovascular diseases on energy metabolism, and are also an experimental justification for the practical choice of these drugs in the complex therapy of CHF.

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References

1. Gaziano, T.A.; Bitton, A.; Anand, S.; Abrahams-Gessel, S.; Murphy, A. Growing epidemic of coronary heart disease in low- and middle-income countries. *Curr Probl Cardiol.* **2010**, *35(2)*, 72-115. doi: 10.1016/j.cpcardiol.2009.10.002.
2. Vaduganathan, M.; Mensah, G.A.; Turco, J.V.; Fuster, V.; Roth, G.A. The Global Burden of Cardiovascular Diseases and Risk: A Compass for Future Health. *J Am Coll Cardiol.* **2022**, *80(25)*, 2361-2371. doi: 10.1016/j.jacc.2022.11.005.
3. Savarese, G.; Becher, P.M.; Lund, L.H.; Seferovic, P.; Rosano, G.M.C.; Coats, A.J.S. Global burden of heart failure: a comprehensive and updated review of epidemiology. *Cardiovasc Res.* **2023**, *118(17)*, 3272-3287. doi: 10.1093/cvr/cvac013.
4. Bragazzi, N.L.; Zhong, W.; Shu, J.; Abu Much, A.; Lotan, D.; Grupper, A.; Younis, A.; Dai, H. Burden of heart failure and underlying causes in 195 countries and territories from 1990 to 2017. *Eur J Prev Cardiol.* **2021**, *28(15)*, 1682-1690. doi: 10.1093/eurjpc/zwaa147.

5. Sapna, F.; Raveena, F.; Chandio, M.; Bai, K.; Sayyar, M.; Varrassi, G.; Khatri, M.; Kumar, S.; Mohamad, T. Advancements in Heart Failure Management: A Comprehensive Narrative Review of Emerging Therapies. *Cureus*. **2023**, *15(10)*, 46486. doi: 10.7759/cureus.46486.
6. Ramani, G.V.; Uber, P.A.; Mehra, M.R. Chronic heart failure: contemporary diagnosis and management. *Mayo Clin Proc*. **2010**, *85(2)*, 180-95. doi: 10.4065/mcp.2009.0494.
7. MacDonald, M.R.; Tay, W.T.; Teng, T.K.; Anand, I.; Ling, L.H.; Yap, J.; Tromp, J.; Wander, G.S.; Naik, A.; Ngarmukos, T.; Siswanto, B.B.; Hung, C.L.; Richards, A.M.; Lam, C.S.P. Regional Variation of Mortality in Heart Failure With Reduced and Preserved Ejection Fraction Across Asia: Outcomes in the ASIAN-HF Registry. *J Am Heart Assoc.* **2020,** *9(1)*, 012199. doi: 10.1161/JAHA.119.012199.. Erratum in: *J Am Heart Assoc*. **2020**, *9(5)*, 014512. doi: 10.1161/JAHA.119.014512.
8. Ponikowski, P.; Voors, A.A.; Anker, S.D.; Bueno, H.; Cleland, J.G.F.; Coats, A.J.S.; Falk, V.; González-Juanatey, J.R.; Harjola, V.P.; Jankowska, E.A.; Jessup, M.; Linde, C.; Nihoyannopoulos, P.; Parissis, J.T.; Pieske, B.; Riley, J.P.; Rosano, G.M.C.; Ruilope, L.M.; Ruschitzka, F.; Rutten, F.H.; van der Meer, P. ESC Scientific Document Group. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J*. **2016**, *37(27)*, 2129-2200. doi: 10.1093/eurheartj/ehw128. Erratum in: *Eur Heart J.* **2018**, *39(10)*, 860. doi: 10.1093/eurheartj/ehw383.
9. Writing Committee Members; ACC/AHA Joint Committee Members. 2022 AHA/ACC/HFSA Guideline for the Management of Heart Failure. *J Card Fail.* **2022**, *28(5)*, 1-167. doi: 10.1016/j.cardfail.2022.02.010.
10. Berliner, D.; Hänselmann, A.; Bauersachs, J. The Treatment of Heart Failure with Reduced Ejection Fraction. *Dtsch Arztebl Int***. 2020**, *117(21)*, 376-386. doi: 10.3238/arztebl.2020.0376.
11. van der Horst, I.C.; Voors, A.A.; van Veldhuisen, D.J. Treatment of heart failure with ACE inhibitors and beta-blockers: what is next? Aldosterone receptor antagonists? *Clin Res Cardiol*. **2007**, *96(4)*, 193-5. doi: 10.1007/s00392-007-0487-y.
12. Dézsi, C.A.; Szentes, V. The Real Role of β-Blockers in Daily Cardiovascular Therapy. *Am J Cardiovasc Drugs*. **2017**, *17(5)*, 361-373. doi: 10.1007/s40256-017-0221-8.
13. Egan, B.M.; Basile, J.; Chilton, R.J.; Cohen, J.D. Cardioprotection: the role of beta-blocker therapy. *J Clin Hypertens (Greenwich).* **2005**, *7(7)*, 409-16. doi: 10.1111/j.1524-6175.2005.04486.x.
14. do Vale, G.T.; Ceron, C.S.; Gonzaga, N.A.; Simplicio, J.A.; Padovan, J.C. Three Generations of β-blockers: History, Class Differences and Clinical Applicability. *Curr Hypertens Rev*. **2019**, *15(1)*, 22-31. doi: 10.2174/1573402114666180918102735.
15. Fisker, F.Y.; Grimm, D.; Wehland, M. Third-generation beta-adrenoceptor antagonists in the treatment of hypertension and heart failure. *Basic Clin Pharmacol Toxicol*. **2015,** *117(1)*, 5-14. doi: 10.1111/bcpt.12396.
16. Oliver, E.; Mayor, F.Jr.; D'Ocon, P. Beta-blockers: Historical Perspective and Mechanisms of Action. *Rev Esp Cardiol (Engl Ed)*, **2019**, *72(10)*, 853-862. doi: 10.1016/j.rec.2019.04.006.
17. Mazur, I.; Belenichev, I.; Kucherenko, L.; Bukhtiyarova, N.; Puzyrenko, A.; Khromylova, O.; Bidnenko, O.; Gorchakova, N. Antihypertensive and cardioprotective effects of new compound 1-(β-phenylethyl)-4-amino-1,2,4-triazolium bromide (Hypertril). *Eur J Pharmacol*. **2019**, *853*, 336-344. doi: 10.1016/j.ejphar.2019.04.013.
18. Bak, P.G.; Belenichev, I.F.; Kucherenko, L.I.; Abramov, A.V.; Khromylova, O.V. Morpho-functional indicators changes of rats' myocardium in experimental doxorubicin-induced chronic heart failure and its pharmacological modulation with new 4-amino-1,2,4-triazole derivative. *Pharmacia.* **2021**, *68(4)*, 919–925. doi: 10.3897/pharmacia.68.e75298.
19. Goncharov, O.; Belenichev, I.; Abramov, A.; Popazova, O.; Kucherenko, L.; Bukhtiyarova, N.; Pavliuk, I. Influence of experimental heart failure therapy with different generations of β-adrenergic blockers on Cardiac Electrical Activity (ECG) and Autonomic Regulation of Heart Rhythm (ARHR). *Pharmacia*. **2023,** *70(4),* 1157–1165. doi: 10.3897/pharmacia.70.e110924.
20. Liu, M.; Lv, J.; Pan, Z.; Wang, D.; Zhao, L.; Guo, X. Mitochondrial dysfunction in heart failure and its therapeutic implications. *Front Cardiovasc Med.* **2022**, *9*, 945142. doi: 10.3389/fcvm.2022.945142.
21. Zhou, B.; Tian, R. Mitochondrial Dysfunction in Heart Failure: Causes, Consequences, and Therapeutic Opportunities. *Cell Metab*. **2021**, *33(1),* 231-243. doi: 10.1016/j.cmet.2020.11.016.
22. Seydi, E.; Tabbati, Y.; Pourahmad, J. Toxicity of Atenolol and Propranolol on Rat Heart Mitochondria. *Drug Res (Stuttg)*. **2020**, *70(4)*, 151-157. doi: 10.1055/a-1112-7032.
23. Brohée, L.; Peulen, O.; Nusgens, B.; Castronovo, V.; Thiry, M.; Colige, A.C.; Deroanne, C.F. Propranolol Sensitizes Prostate Cancer Cells to Glucose Metabolism Inhibition and Prevents Cancer Progression. *Sci Rep. 2018*, *8(1)*, 7050. doi: 10.1038/s41598-018-25340-9.
24. Cicek, F.A.; Toy, A.; Tuncay, E.; et al. Beta-Blocker Timolol Alleviates Hyperglycemia-Induced Cardiac Damage via Inhibition of Endoplasmic Reticulum Stress. *J Bioenerg Biomembr*. **2014**, *46*, 377-387. doi: 10.1007/s10863-014-9568-6.

25. Cocco, T.; Cutecchia, G.; Montedoro, G.; Lorusso, M. The Antihypertensive Drug Carvedilol Inhibits the Activity of Mitochondrial NADH-Ubiquinone Oxidoreductase. *J Bioenerg Biomembr.* **2002**, *34(4)*, 251-258. doi: 10.1023/a:1020248300766.
26. Belenichev, I.F.; Bak, P.G.; Popazova, O.O.; Bukhtiyarova, N.; Yadlovsky, O.E. Nitric Oxide-Dependent Mechanism of Endothelial Dysfunction Formation: A Promising Target for Pharmacological Management. *Biopolymers and Cell.* **2022**, *38*, 145-157. doi: 10.7124/bc.000A79.
27. Chekman, I.S.; Belenichev, I.F.; Kucherenko, L.I.; Mazur, I.A.; Nagornaia, E.A.; Bukhtiiarova, N.V.; Parniuk, N.V. NO-Dependent Mechanisms of Cardioprotective Activity of MT Preparation During Course Administration to SHR Rats. *Eksperimental'naia i Klinicheskaia Farmakologiia*. **2013**, *76(8)*, 24-26.
28. Cosentino, F.; Bonetti, S.; Rudolf, R.; Eto, M.; Werner-Felmayer, G.; Volpe, M.; Lüscher, T.F. Nitric-Oxide-Mediated Relaxations in Salt-Induced Hypertension: Effect of Chronic β1-Selective Receptor Blockade. *J Hypertens*. **2002**, *20(3)*, 421-428.
29. Chen, Y.; Hong, X. Effects of Carvedilol Reduce Conjunctivitis Through Changes in Inflammation, NGF, and VEGF Levels in a Rat Model. *Exp Ther Med.* **2016**, *11(5)*, 1987-1992.
30. Watanabe, K.; Ohta, Y.; Inoue, M.; Ma, M.; Wahed, M.I.; Nakazawa, M.; Hasegawa, G.; Naito, M.; Fuse, K.; Ito, M.; Kato, K.; Hanawa, H.; Kodama, M.; Aizawa, Y. Bisoprolol Improves Survival in Rats with Heart Failure. *J Cardiovasc Pharmacol*. **2001**, *38(1)*, S55-S58.
31. Xie, S.; Xu, S.C.; Deng, W.; Tang, Q. Metabolic Landscape in Cardiac Aging: Insights into Molecular Biology and Therapeutic Implications. *Signal Transduct Target Ther*. **2023**, *8(1)*, 114. doi: 10.1038/s41392-023-01378-8.
32. Nielsen, T.T.; Støttrup, N.B.; Løfgren, B.; Bøtker, H.E. Metabolic Fingerprint of Ischaemic Cardioprotection: Importance of the Malate–Aspartate Shuttle. *Cardiovasc Res.* **2011**, *91(3)*, 382-391. doi: 10.1093/cvr/cvr051.
33. Lu, M.; Zhou, L.; Stanley, W.C.; Cabrera, M.E.; Saidel, G.M.; Yu, X. Role of the Malate-Aspartate Shuttle on the Metabolic Response to Myocardial Ischemia. *J Theor Biol.* **2008**, *254(2)*, 466-475. doi: 10.1016/j.jtbi.2008.05.033.
34. Kiyuna, L.A.; Albuquerque, R.P.E.; Chen, C.H.; Mochly-Rosen, D.; Ferreira, J.C.B. Targeting Mitochondrial Dysfunction and Oxidative Stress in Heart Failure: Challenges and Opportunities. *Free Radic Biol Med.* **2018**, *129*, 155-168. doi: 10.1016/j.freeradbiomed.2018.09.019.
35. Gallo, G.; Rubattu, S.; Volpe, M. Mitochondrial Dysfunction in Heart Failure: From Pathophysiological Mechanisms to Therapeutic Opportunities. *Int J Mol Sci.* **2024**, *25(5)*, 2667. doi: 10.3390/ijms25052667.
36. Kamenshchyk, A.; Belenichev, I.; Oksenych, V.; Kamyshnyi, O. Combined Pharmacological Modulation of Translational and Transcriptional Activity Signaling Pathways as a Promising Therapeutic Approach in Children with Myocardial Changes. *Biomolecules.* **2024**, *14*, 477. doi: 10.3390/biom14040477.
37. Zong, Y.; Li, H.; Liao, P.; et al. Mitochondrial Dysfunction: Mechanisms and Advances in Therapy. *Sig Transduct Target Ther*. **2024**, *9*, 124. doi: 10.1038/s41392-024-01839-8.
38. Nguyen, B.Y.; Ruiz-Velasco, A.; Bui, T.; Collins, L.; Wang, X.; Liu, W. Mitochondrial Function in the Heart: Insight into Mechanisms and Therapeutic Potentials. *Br J Pharmacol.* **2019**, *176(22)*, 4302-4318. doi: 10.1111/bph.14431.
39. Rosca, M.G.; Tandler, B.; Hoppel, C.L. Mitochondria in Cardiac Hypertrophy and Heart Failure. *J Mol Cell Cardiol*. **2013**, *55*, 31-41. doi: 10.1016/j.yjmcc.2012.09.002.
40. Bedi, K.C. Jr.; Snyder, N.W.; Brandimarto, J.; Aziz, M.; Mesaros, C.; Worth, A.J.; Wang, L.L.; Javaheri, A.; Blair, I.A.; Margulies, K.B.; Rame, J.E. Evidence for Intramyocardial Disruption of Lipid Metabolism and Increased Myocardial Ketone Utilization in Advanced Human Heart Failure. *Circulation*. **2016**, *133(8)*, 706-716. doi: 10.1161/CIRCULATIONAHA.115.017545.
41. Huss, J.M.; Kelly, D.P. Mitochondrial Energy Metabolism in Heart Failure: A Question of Balance. *J Clin Invest*. **2005**, *115(3)*, 547-555. doi: 10.1172/JCI24405.
42. Belenichev, I.; Popazova, O.; Bukhtiyarova, N.; Savchenko, D.; Oksenych, V.; Kamyshnyi, O. Modulating Nitric Oxide: Implications for Cytotoxicity and Cytoprotection. *Antioxidants (Basel)*. **2024**, *13(5)*, 504. doi: 10.3390/antiox13050504.
43. Belenichev, I.; Aliyeva, O.; Popazova, O.; Bukhtiyarova, N. Molecular and Biochemical Mechanisms of Diabetic Encephalopathy. *Acta Biochim Pol*. **2023**, *70(4)*, 751-760. doi: 10.18388/abp.2020_6953.
44. Rosca, M.G.; Tandler, B.; Hoppel, C.L. Mitochondria in Cardiac Hypertrophy and Heart Failure. *J Mol Cell Cardiol.* **2013**, *55*, 31-41. doi: 10.1016/j.yjmcc.2012.09.002.
45. Jain, M.; Rivera, S.; Monclus, E.A.; Synenki, L.; Zirk, A.; Eisenbart, J.; Feghali-Bostwick, C.; Mutlu, G.M.; Budinger, G.R.; Chandel, N.S. Mitochondrial Reactive Oxygen Species Regulate Transforming Growth Factor-β Signaling. *J Biol Chem.* **2013**, *288(2)*, 770-777. doi: 10.1074/jbc.M112.431973.
46. Belenichev, I.; Bak, P.; Popazova, O.; Ryzhenko, V.; Bukhtiyarova, N.; Puzyrenko, A. Integrative and Biochemical Parameters in Rats in the Simulation of Doxorubicin Chronic Heart Failure and During the Use of β-Adrenergic Blockers. *J Fac Pharm Ankara Univ.* **2023**, *47(1)*, 228-238. doi: 10.33483/jfpau.1131302.
47. Almannai, M.; El-Hattab, A.W. Nitric Oxide Deficiency in Mitochondrial Disorders: The Utility of Arginine and Citrulline. *Front Mol Neurosci*. **2021**, *14*, 682780. doi: 10.3389/fnmol.2021.682780.

48. Litvinova, L.; Atochin, D.N.; Fattakhov, N.; Vasilenko, M.; Zatolokin, P.; Kirienkova, E. Nitric Oxide and Mitochondria in Metabolic Syndrome. *Front Physiol.* **2015**, *6*, 20. doi: 10.3389/fphys.2015.00020.
49. Sgobbo, P.; Pacelli, C.; Grattagliano, I.; Villani, G.; Cocco, T. Carvedilol Inhibits Mitochondrial Complex I and Induces Resistance to H2O2-Mediated Oxidative Insult in H9C2 Myocardial Cells. *Biochim Biophys Acta.* **2007**, *1767(3)*, 222-232. doi: 10.1016/j.bbabio.2007.01.023.
50. Santos, D.L.; Moreno, A.J.; Leino, R.L.; Froberg, M.K.; Wallace, K.B. Carvedilol Protects Against Doxorubicin-Induced Mitochondrial Cardiomyopathy. *Toxicol Appl Pharmacol.* **2002**, *185(3)*, 218-227. doi: 10.1006/taap.2002.9532.
51. Oliveira, P.J.; Marques, M.P.; Batista de Carvalho, L.A.; Moreno, A.J. Effects of Carvedilol on Isolated Heart Mitochondria: Evidence for a Protonophoretic Mechanism. *Biochem Biophys Res Commun*. **2000**, *276(1)*, 82-87. doi: 10.1006/bbrc.2000.3374.
52. Diogo, C.V.; Deus, C.M.; Lebiedzinska-Arciszewska, M.; Wojtala, A.; Wieckowski, M.R.; Oliveira, P.J. Carvedilol and Antioxidant Proteins in a Type I Diabetes Animal Model. *Eur J Clin Invest*. **2017**, *47(1)*, 19-29. doi: 10.1111/eci.12696.
53. Zhu, B.Q.; Simonis, U.; Cecchini, G.; Zhou, H.Z.; Li, L.; Teerlink, J.R.; Karliner, J.S. Comparison of Pyrroloquinoline Quinone and/or Metoprolol on Myocardial Infarct Size and Mitochondrial Damage in a Rat Model of Ischemia/Reperfusion Injury. *J Cardiovasc Pharmacol Ther*. **2006**, *11(2)*, 119-128. doi: 10.1177/1074248406288757.
54. Power, A.S.; Norman, R.; Jones, T.L.M.; Hickey, A.J.; Ward, M.L. Mitochondrial Function Remains Impaired in the Hypertrophied Right Ventricle of Pulmonary Hypertensive Rats Following Short Duration Metoprolol Treatment. *PLoS One*. **2019**, *14(4)*, 0214740. doi: 10.1371/journal.pone.0214740.
55. Wang, P.; Zaragoza, C.; Holman, W. Sodium-Hydrogen Exchange Inhibition and Beta-Blockade Additively Decrease Infarct Size. *Ann Thorac Surg*. **2007**, *83(3)*, 1121-1127. doi: 10.1016/j.athoracsur.2006.10.03.
56. Omerovic, E.; Bollano, E.; Soussi, B.; Waagstein, F. Selective Beta1-Blockade Attenuates Post-Infarct Remodelling Without Improvement in Myocardial Energy Metabolism and Function in Rats with Heart Failure. *Eur J Heart Fail*. **2003**, 5(6), 725-32. doi: 10.1016/s1388-9842(03)00153-3.
57. Metra, M.; Nodari, S.; Bordonali, T.; Milani, P.; Lombardi, C.; Bugatti, S.; Fontanella, B.; Verzura, G.; Danesi, R.; Dei Cas, L. Bisoprolol in the Treatment of Chronic Heart Failure: From Pathophysiology to Clinical Pharmacology and Trial Results. *Ther Clin Risk Manag*. **2007**, *3(4)*, 569-578.
58. Laser, A.; Neubauer, S.; Tian, R.; Hu, K.; Gaudron, P.; Ingwall, J.S.; Ertl, G. Long-Term Beta-Blocker Treatment Prevents Chronic Creatine Kinase and Lactate Dehydrogenase System Changes in Rat Hearts After Myocardial Infarction. *J Am Coll Cardiol*. **1996**, *27(2)*, 487-493. doi: 10.1016/0735-1097(95)00458-0.
59. Ichihara, S.; Yamada, Y.; Ichihara, G.; Kanazawa, H.; Hashimoto, K.; Kato, Y.; Matsushita, A.; Oikawa, S.; Yokota, M.; Iwase, M. Attenuation of Oxidative Stress and Cardiac Dysfunction by Bisoprolol in an Animal Model of Dilated Cardiomyopathy. *Biochem Biophys Res Commun*. **2006**, *350(1)*, 105-113. doi: 10.1016/j.bbrc.2006.09.026.
60. Nuevo-Tapioles, C.; Santacatterina, F.; Stamatakis, K.; et al. Coordinate β-Adrenergic Inhibition of Mitochondrial Activity and Angiogenesis Arrest Tumor Growth. *Nat Commun*. **2020**, *11*, 3606. doi: 10.1038/s41467-020-17384-1.
61. Seleme, V.B.; Marques, G.L.; Mendes, A.E.M.; Rotta, I.; Pereira, M.; Júnior, E.L.; da Cunha, C.L.P. Nebivolol for the Treatment of Essential Systemic Arterial Hypertension: A Systematic Review and Meta-Analysis. *Am J Cardiovasc Drugs*. **2021**, *21(2)*, 165-180. doi: 10.1007/s40256-020-00422-0.
62. Bhadri, N.; Razdan, R.; Goswami, S.K. Nebivolol, a β-Blocker Abrogates Streptozotocin-Induced Behavioral, Biochemical, and Neurophysiological Deficit by Attenuating Oxidative-Nitrosative Stress: A Possible Target for the Prevention of Diabetic Neuropathy. *Naunyn Schmiedebergs Arch Pharmacol*. **2018**, *391(2)*, 207-217. doi: 10.1007/s00210-017-1450-8.
63. Gul, R.; Alsalman, N.; Bazighifan, A.; Alfadda, A.A. Comparative Beneficial Effects of Nebivolol and Nebivolol/Valsartan Combination Against Mitochondrial Dysfunction in Angiotensin II-Induced Pathology in H9c2 Cardiomyoblasts. *J Pharm Pharmacol*. **2021**, *73(11)*, 1520-1529. doi: 10.1093/jpp/rgab124.
64. Chen, Q.; Jiang, H.; Wang, Z.; Cai, L.Y.; Jiang, Y.C.; Xie, L.; Zhou, Y.; Zeng, X.; Ji, N.; Shen, Y.Q.; Chen, Q.M. Adrenergic Blockade by Nebivolol to Suppress Oral Squamous Cell Carcinoma Growth via Endoplasmic Reticulum Stress and Mitochondria Dysfunction. *Front Pharmacol*. **2021**, *12*, 691998. doi: 10.3389/fphar.2021.691998.
65. Bețiu, A.M.; Noveanu, L.; Hâncu, I.M.; Lascu, A.; Petrescu, L.; Maack, C.; Elmér, E.; Muntean, D.M. Mitochondrial Effects of Common Cardiovascular Medications: The Good, the Bad, and the Mixed. *Int J Mol Sci*. **2022**, *23(21)*, 13653. doi: 10.3390/ijms232113653.