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ORIGINAL ARTICLE / ÖZGÜN MAKALE

INTEGRATIVE AND BIOCHEMICAL PARAMETERS IN RATS IN THE SIMULATION OF DOXORUBICIN CHRONIC HEART FAILURE AND DURING THE USE OF B-ADRENERGIC BLOCKERS

DOKSORUBİSİN İLE OLUŞTURULAN KRONİK KALP YETMEZLİĞİ MODELİNDE VE B-ADRENERJİK BLOKERLERİN KULLANIMI SIRASINDA SIÇANLARDA BÜTÜNLEYİCİ VE BİYOKİMYASAL PARAMETRELER

Igor BELENICHEV[1], Pavlo BAK[1], Olena POPAZOVA[2]*, Victor RYZHENKO[3], Nina BUKHTIYAROVA[4], Andrii PUZYRENKO[5]

[1]Zaporizhzhia State Medical University, Department of Pharmacology and Medical Formulation with Course of Normal Physiology, 26 Mayakovsky Ave., Zaporizhzhia 69000 Ukraine

[*2]Zaporizhzhia State Medical University, Department of Histology, Cytology and Embryology, 26 Mayakovsky Ave., Zaporizhzhia 69000 Ukraine

[3]Zaporizhzhia State Medical University, Department of Medical and Pharmaceutical Informatics and Advanced Technologies, Zaporizhzhia State Medical University26 Mayakovsky Ave., Zaporizhzhia 69000 Ukraine

[4]Zaporizhzhia State Medical University, Department of Clinical Laboratory Diagnostics, Zaporizhzhia State Medical University, 26 Mayakovsky Ave., Zaporizhzhia 69000 Ukraine

[5]Pathology and Laboratory Medicine, College of Wisconsin, Milwaukee, United States

ABSTRACT

Objective: *In the treatment of chronic heart failure, β-blockers are actively used - Carvedilol, Nebivolol, Metoprolol, Bisoprolol, etc. However, they have a number of serious adverse reactions, and their therapeutic efficacy does not always meet the needs of the clinic. All this prompted the creation of a new potential drug Hypertril (bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazolium. We*

* **Corresponding Author / Sorumlu Yazar:** Olena Popazova
e-mail / e-posta: popazova.ea@gmail.com, **Phone / Tel.:** +3804726250

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aimed to conduct a comparative assessment of β-blockers of different generations and "Hypertril" in the conditions of modeling the doxorubicin model of chronic heart failure (CHF) in terms of the effect on biochemical markers of myocardial damage and integrative parameters.

Material and Method: *CHF was modeled on 85 white outbred rats weighing 190–220 g by administering doxorubicin at a total dose of 15 mg/kg, nebivolol (10 mg/kg), carvedilol (50 mg/kg), bisoprolol (10 mg/kg), metoprolol (15 mg/kg) and hypertril (3.5 mg/kg) were administered intragastrically once a day as a suspension 1% starchy mucus for 30 days after 14 days of doxorubicin administration. The cardioprotective effect of drugs was assessed by improving integrative parameters (survival, heart mass index, severity in points) and by normalizing cardiospecific markers (NT-proBNP, D-dimer, eNOS, MB-CPK, and ST2).*

Result and Discussion: *The introduction of Hypertril not only prolonged the life of animals with CHF in comparison with the reference drugs, but also prevented early death and contributed to a decrease in the severity of symptoms (hydrothorax, ascites, scrotal edema). The administration of hypertril to rats with CHF led to a decrease in mortality, a decrease in the heart mass index, in the blood of the main cardiospecific markers to the values of intact animals, and also led to an increase in expression of eNOS, which testified to its significant cardioprotective effect with NO-mimetic effect. The obtained results demonstrated the undoubted advantage of Hypertril over the basic β-adrenergic blockers and experimentally substantiated further in-depth studies to create a drug based on it for the treatment of CHF.*

Keywords: *Bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazolium, cardioprotection, chronic heart failure, hypertril, endothelial dysfunction, metoprolol, β-blockers*

ÖZ

Amaç: *Kronik kalp yetmezliğinin tedavisinde karvedilol, nebivolol, metoprolol, bisoprolol vb. β-blokerler aktif olarak kullanılmaktadır. Bununla birlikte, bir dizi ciddi yan etkileri bulunmakta olup tedavi edici etkinlikleri her zaman kliniğin ihtiyaçlarını karşılamamaktadır. Bütün bunlar yeni bir potansiyel ilacın (Hypertril, bromür 1-(β-feniletil)-4-amino-1,2,4-triazolyum)) geliştirilmesine yol açmaktadır. Farklı nesil β-blokerlerin ve Hipetrilin farkli koşullar altında (miyokard hasarının biyokimyasal belirteçleri ve integratif parametreler üzerindeki etkisi açısından kronik kalp yetmezliğinin (KKY) doksorubisin modellenmesiyle) karşılaştırmalı bir değerlendirmesinin yapılması amaçlanmıştır.*

Gereç ve Yöntem: *KKY, doksorubisin toplam 15 mg/kg dozunda uygulanarak 190-220 gram ağırlığındaki 85 beyaz fare üzerinde modellenmiştir. Nebivolol (10 mg/kg), karvedilol (50 mg/kg), bisoprolol (10 mg/kg), metoprolol (15 mg/kg) ve Hipertril (3.5 mg/kg) 14 günlük doksorubisin uygulamasından sonra 30 gün boyunca % 1 nişastalı mukus süspansiyonu olarak günde bir kez intragastrik olarak uygulanmıştır. İlaçların kardiyoprotektif etkisi, integratif parametrelerin iyileştirilmesi (sağkalım, kalp kütle indeksi, noktalardaki şiddet) ve kardiyospesifik belirteçlerin normalleştirilmesiyle (NT-proBNP, D-dimer, eNOS, MB-CPK ve ST2) değerlendirilmiştir.*

Sonuç ve Tartışma: *Hipertril'in tanıtılması, referans ilaçlara kıyasla KKY'li hayvanların ömrünü uzatmakla kalmamış, aynı zamanda erken ölümü önlemiş ve belirtilerin şiddetinin (hidrotoraks, asit, skrotal ödem) azalmasına katkıda bulunmuştur. KKY'li farelere hipertril uygulanması, ölüm oranında azalışa, kalp kitle indeksinde düşüşe ve ayrıca NO-mimetik etki ile önemli kardiyoprotektif etkisine tanıklık eden eNOS ifadesinde bir artışa yol açmıştır. Elde edilen sonuçlar, Hypertril'in temel β-adrenerjik blokerlerin üzerindeki tartışmasız avantajını göstermiştir ve KYY tedavisi için buna dayalı bir ilaç oluşturmak amacıyla deneysel olarak derinlemesine çalışmalar yapılmıştır.*

Anahtar Kelimeler: *Bromür 1-(β-feniletil)-4-amino-1,2,4-triazolyum), endotel disfonksiyonu, hipertril, kardiyoproteksiyon, kronik kalp yetmezliği, metoprolol, β-blokerler*

INTRODUCTION

Chronic heart failure (CHF) is one of the alarming problem of modern cardiology worldwide due to its high prevalence, steady increase in the number of cases all over the world, frequent readmissions, poor quality of treatment, high level of disability and mortality of patients, and increased treatment costs [1]. World statistics are disappointing: the current prevalence of clinically significant chronic heart failure in the general population is at least 1.8-2.0%; among people over 65 years of age, the incidence of CHF increases to 6-10%, and decompensation becomes the most frequent cause for hospitalization

[2]. The frequency of patients with asymptomatic left ventricular dysfunction is at least 4 times higher than the number of patients with clinically expressed CHF [3]. Over the past 15 years, the number of hospitalized patients diagnosed with CHF has tripled, and over the past 40 years, the value has increased by 6 times [4]. For over a decade, β-adrenergic blockers have actively been used in the treatment of heart failure. These drugs reduce excessive sympathetic stimulation of the myocardium, desensitizes myocardial β1-adrenoreceptors, reduces the calcium overload of cardiomyocytes, reduces heart's oxygen demand, inhibit lipid peroxidation and stabilize cell membranes, and have an antiarrhythmic effect [5]. The use of β-blockers can improve the survival rates of patients by effectively increasing the ejection fraction and reducing the mass and sphericity of the left ventricle of the heart. Early initiation of pharmacotherapy with B-blockers may prevent/decelerate or even induce reversal of cardiac remodeling [6]. The leading component of this effect of β-blockers is their cardioprotective properties. Drugs such as Metoprolol, Carvedilol, Bisoprolol and Nebivolol are used in CHF. From the standpoint of evidence-based medicine, efficacy in CHF has been confirmed only in some representatives of these classes. Possessing a different set of characteristics, drugs can have variable effect on the survival of patients with CHF and on cardioprotection.

The search for optimal remedies for treating CHF, as well as methods and ways of inhibiting CR, is constantly ongoing. The foregoing served as a rationale for the creation of a new drug of the original structure (bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazolium, working title Hypertril) which has NO-mimetic, β_1-adrenergic blocking, antihypertensive, anti-ischemic action and belongs to the class IV of toxicity (LD50 is 683.4 mg/kg with intragastric administration to rats) [7-9]. SPA “Farmatron” together with the scientific and technological complex “Institute of Single Crystals” of the NAS of Ukraine developed a laboratory methods and technological formulas for the synthesis of the substance and the production of ampoule solutions Hypertril substance, for which standardization was carried out (certificate №2, series 020213). 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.

The aim of the research was to conduct a comparative assessment of the effectiveness of Nebivolol, Carvedilol, Bisoprolol, Metoprolol succinate and a new potential drug "Hypertril" in the conditions of modeling doxorubicin CHF to improve integrative and biochemical parameters.

MATERIAL AND METHOD

Animals

The experiments were carried out on 85 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).

Experimental Model

Doxorubicin model was used to reproduce chronic heart failure [10]. 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 ventricular

myocardial contractility, its eccentric remodeling, and the formation of progressive CHF in rats [10,24,26].

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 [7], Metoprolol succinate - 15 mg/kg [9,11], Nebivolol 10 mg/kg [12], Carvedilol 50 mg/kg [13], Bisoprolol 10 mg/kg [3]. 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), Metoprolol succinate tablets (Astra Zeneca UK Ltd, Sweden), Nebivolol tablets (Teva Pharmaceutical Industries, Ltd, Israel), Carvedolol tablets (Salutas Pharma GmbH, Germany), Bisoprolol tablets (Teva Pharmaceutical Industries, Ltd, Israel).

Anesthesia

At the end of the experiment, in animals under anesthesia (sodium ethaminal, 40 mg/kg), the heart was taken and weighed, and blood was taken from the abdominal artery with a syringe for biochemical studies.

Pharmacological and Physiological Methods

The animals were examined daily and clinical symptoms of CHF were recorded - hydrothorax, ascites, hepatomegaly, scrotum edema, animal weight, mortality. Motor activity was studied in the open field test (arena with dimensions of 80x80x35cm). The image was captured and recorded using a SSC-DC378P color video camera (Sony, Japan). At the end of the experiment, the heart was taken from the animals, the blood was removed and weighed on an analytical balance AXIS ANG220C.

Biochemical and Enzyme Immunoassays Methods

The blood was centrifuged (3000 rpm, 25 min at 50^0C (Eppendorf, Germany). In the blood serum, activity of total CPK (creatine phosphokinase) and cardiac isoenzyme (MB-CPK) is observed according to the High Technology Inc. (USA). Spectrophotometer BioSpectrometer kinetic, Ependorff (Germany). Also, the molecular marker of myocardial damage ST2 protein was determined in the blood serum by the solid-phase immunosorbent sandwich ELISA method using the Critical Diagnostics Presage® ST2 Assay kit (REF# BC-1065). The activity of endothelial NO-synthase (eNOS) was determined in serum by enzyme immunoassay (Cloud-Clone Corporation kit, USA) (#PAA868Ra01), as well as D- dimer (Vector-Best kit, Russia), NT-proBNP (Vector-Best kit, Russia), (enzyme immunoassay analyzer – Immunochem-2200, USA). C-reactive protein was determined by the immunoturbodimetric method (Cormay kit, biochemical analyzer ACCENT-200, Poland).

Statistical Methods

The results of the study were calculated using a standard statistical package «STATISTICA® for Windows 6.0» (StatSoftInc., №AXXR712D833214FAN5), «SPSS 16.0» and«Microsoft Office Excell 2003». To determine the presence and nature of the relationship between numerical variables, a regression analysis procedure was used using linear, logarithmic, power, exponential, polynomial (second and third degree) models, achieving an independent (according to the Durbin-Watson criterion), normal distribution of residuals (at the same time as skewness and kurtosis values were used for the goodness of fit criterion. Distribution normality was assessed using the Shapiro-Wilk test. The data were presented as an average value. The significance of negativity between the mean values was determined by Student's t-test (in the case of a normal distribution). The Mann-Whitney U-test was used in the case of a distribution that is negative compared to normal or analysis of ordinal variables. To compare independent variables in more than two samples, analysis of variance (ANOVA) was used with a normal distribution or the Kruskal-Wallis test for a distribution that was negative from normal. Negativity $p<0.05$ (95%) was considered statistically significant for all types of analysis.

RESULT AND DISCUSSION

The introduction of doxorubicin over the next 14 days (day 45) led to the formation of clinically confirmed heart failure (hydrothorax, ascites, hepatomegaly, scrotal edema occurred in 100% of rats). In the control group, from st to 45th days of observation, 70% of the animals died (Table 1). So, in the control group, 6 rats died on day 20, 4 rats on day 21, 3 rats – on day 27, and 1 – on day 29 of the experiment. In the group of animals with CHF treated with Hypertril at a dose of 3.5 mg/kg intragastrically, 1 rat died on day 40. In the group of animals with CHF treated with Metoprolol at a dose of 15 mg/kg, 3 rats died on day 21, and 1 rat died on day 24, and then 3 rats died on day 32 of observation. On the 29th day of observation, 1 rat died in the group of animals treated with Metoprolol. Analysis of the results of treatment of experimental CHF in rats with Hypertril, Metoprolol, Nebivolol, Carvedilol and Bisoprolol indicated that all the studied drugs significantly increased the survival rate of animals, however, during the experiment, when Hypertril was administered, more animals survived. All drugs significantly lengthened the lifespan of rats compared to the control, but by the 45th day of the experiment, Metoprolol and Biosprolol increased by 60%, Carvedilol – by 50%, Nebivolol – by 80%, Hypertril – by 95% (Table 1). The use of Hypertril not only prolonged the life of animals compared to the reference drugs, but also prevented early death (3 weeks of observation) of rats with experimental CHF.

Table 1. Influence of Hypertril on the survival of animals with CHF at different periods of the experiment

Group of animals	Survival at 21 days	% Survival at 21 days	Survival at 45 days	% Survival at 45 days
Intact	10/10	100	10/10	100
CHF (control)	20/10	50	20/6	30
CHF + Hypertril, 3.5 mg/kg	20/20	100*[1]	20/19	95*
CHF + Metoprolol succinate, 15mg/kg	20/16	80*	20/12	60*
CHF + Bisoprolol, 10 mg/kg	20/15	80*	20/12	60*
CHF + Carvedilol, 50 mg/kg	20/16	80*	20/10	50*
CHF + Nebivolol, 10 mg/kg	20/19	80*	20/16	80*

Note: * - $p<0.05$ is in relation to the indicators of the control group

Observation of animals of the control group showed that they are inactive, the number of motor acts decreased from 34.2 to 17.6. The administration of Hypertril at a dose of 3.5 mg/kg to animals with CHF led to a significant increase in motor activity by 71% on the 21st day of observation and by 81% on the 45th day. The introduction of studies into comparison did not lead to such an analysis of experimental CHF. The appointment of Hypertril also contributed to a decrease in the number of animals with severe symptoms (hydrothorax, ascites, swelling of the scrotum). So, in this experimental group, only 26% of the animals on the 45th day had these signs (against 100% in the control group). In groups of animals treated with metoprolol - 50%, bisoprolol - 42%, carvedilol and metoprolol - 50%, Nebivolol - 31% (Table 2).

The study of the heart mass index (Table 3) shows that myocardial hypertrophy developed in rats with doxorubicin induced CHF, which was recorded on the 45th day of the experiment. The index of hypertrophy (percentage of heart weight to animal body weight) in experimental animals increased from 0.401 ± 0.015 to 0.676 ± 0.011 on day 45 ($p<0.05$). The administrationof "Hypertril" slowed down the development of hypertrophy, when this indicator was recorded on the 45th day of observation. The heart mass index in the rats treated with Hypertril was 0.462 ± 0.022 and was significantly lower by 31.6% compared to the same indicator in the control group and the group of animals treated with metoprolol

(0.56 ± 0.014). Metoprolol and Bisoprolol significantly reduced myocardial hypertrophy in rats with CHF by 17%, Nebivolol by 29%, Carvedilol by 11%.

Table 2. The influence of Hypertril on the integrative parameters of animals with CHF at different periods of the experiment

Group of animals	The number of motor acts for 21 days (3 min)	The number of animals with severe CHF symptoms on day 21	The number of motor acts for 45 days (3 min)	The number of animals with severe CHF symptoms on day 45
Intact	3.2±8.5	0	37.3±6.8	0
CHF (control)	17.6±3.4	10/10 (100%)	11.7±2.4	6/6 (100%)
CHF + Hypertril, 3.5 mg/kg	30.1±7.2*	20/4 (20,0%)*	21.2±5.8* (+81%)	19/5 (26%)*
CHF + Metoprolol succinate, 15mg/kg	16.7±5.1	16/6(37,5%)*	12.0±1.4 (+2%)	12/6(50%)*
CHF + Bisoprolol, 10 mg/kg	17.5±6.3	15/6(40%)*	12.8±2.7	12/5(42%)*
CHF + Nebivolol, 10 mg/kg	23.0±4.4	18/5(27,7%)*	18.0±3.5	16/5(31%)*
CHF + Carvedilol, 50 mg/kg	17.2±4.0	16/7(43,7%)*	12.0±3.2	10/5 (50%)

Note: * - $p<0.05$ is in relation to the indicators of the control group

Table 3. The effect of Hypertril on body weight and heart mass index of animals with CHF on the 45th day of the experiment

Group of animals	Body weight s on the 1st day, g	Body weight, on the 45th day, g	Weight of the heart on the 45th day, g	Heart mass index on the 45th day
Intact (п=10)	178.0±6.4	206.5±9.8	0.827±0,036	0.401 ± 0.015
CHF (control) (п=6)	182.1±6.2	173.6±4.5	1.172±0,012	0.676 ± 0.011
CHF + Hypertril, 3.5 mg/kg (п=19)	174.2±5.0	177.7±4.0	0.811±0,025	0.462 ± 0.022*
CHF + Metoprolol succinate, 15mg/kg (п=12)	176.6±5.3	171.0±3.6	0.96±0,025	0.56 ± 0.014*
CHF + Bisoprolol,10 mg/kg (п=12)	182.4±6.2	188±4.5	1.065±0,024	0.56± 0.012*
CHF + Nebivolol, 10 mg/kg (п=16)	188.3±5.7	184.6±5,0	1.020±0.017	0.48± 0.011*
CHF + Carvedilol, 50 mg/kg (п=10)	189.7±6.2	208.6±6.7	1.246±0.027	0.597± 0.021

Note: * - $p<0.05$ is in relation to the indicators of the control group

When modeling CHF on day 45, a significant increase in the activity of total creatine phosphokinase (CPK) by 1.4 times and cardiospecific creatine phosphokinase (MB-CPK) by 2 times was recorded in the blood serum of experimental animals, which indicateds ischemic damage to the myocardium. The course administration of the studied preparations to rats with CHF led to a decrease in the activity of CPK and MB-CPK. Thus, Hypertril significantly reduced hyperenzymemia of cardiospecific creatine phosphokinase (MB-CPK) by 44%, and hyperenzymemia of total CPK by 24.8%. At the same time, Hypertril is significantly superior to Metoprolol, Bisoprolol and Carvedilol in terms of the degree of decrease in the activity of MB-CPK. Further analysis of biochemical studies of

the blood serum of animals with CHF revealed a significant increase in C-reactive protein by 3.3 times relative to the parameters of the intact group (Table 4). An increase in C-reactive protein indicates the development of an inflammatory response in modeling CHF and the formation of endothelial dysfunction. The appointment of Hypertril to animals with CHF led to a significant decrease in C-reactive protein by 25.5%, and Nebivolol by 19.1% compared with the control group. The introduction of Metoprolol, Carvedilol and Bisoprolol did not affect the level of C-reactive protein in the blood of animals with CHF (Table 5).

Table 4. The effect of Hypertril on the activity of creatine phosphokinase and the levels of C-reactive protein in the blood serum of animals with CHF on the 45th day of the experiment

Group of animals	MB- CPK, IU / l	Total CPK, IU / l	C-reactive protein, g / l
Intact (n=10)	21.6±2.7	230.2±17.2	1.4±0.12
CHF (control) (n=6)	47.6±3.8	493.6±31.7	4.7±0.38
CHF + Hypertril, 3.5 mg/kg (n=19)	26.7±2.1*	331.8±27.5*	3.5±0.30*
CHF + Metoprolol succinate, 15mg/kg (n=12)	35.8±2.8*	374.5±31.3*	4.7±0.52
CHF + Bisoprolol,10 mg/kg (n=12)	35.7±3.2*	379.1±27.2*	4.8±0.61
CHF + Nebivolol, 10 mg/kg (n=16)	30.2±2.4*	355.7±24.2*	3.8±0.44*
CHF + Carvedilol, 50 mg/kg (n=10)	34.2±3.1	367.2±45.1*	4.0±0.32

Note: * - p<0.05 is in relation to the indicators of the control group

Table 5. Influence of Hypertril on the content of NT-proBNP, D-dimer, eNOS and ST2 protein in the blood serum of animals with CHF on the 45th day of the experiment

Group of animals	NT-proBNP pg/ml	D-dimer ng/ml	eNOS, pg/ml	ST2, ng/ml
Intact (n=10)	201.6±10.0	14.7±0.9	23.3±0,91	20.2±0.6
CHF (control) (n=6)	710.8±13.6	380.8±6.2	8.9±0.30	103.2±2.5
CHF + Hypertril, 3.5 mg/kg (n=19)	218.3±5.4*	14.3±0.9*	73.9±6.58*	23.7±2.6*
CHF + Nebivolol, 10 mg/kg (n=16)	300.0±10.9*	48.8±4.2*	59.3±0.79*	25.5±1.8*
CHF + Bisoprolol,10 mg/kg (n=12)	353.3±9.9*	160.2±16.6*	19.6±1.27*	32.5±1.0*
CHF + Carvedilol, 50 mg/kg (n=10)	458.5±12.5*	72.1±3.5*	14.7±0.80*	59.9±6.0*
CHF + Metoprolol succinate, 15mg/kg (n=12)	478.0±16.8*	66.8±4.5*	9.3±0.52	62.08±3.2*

Note: * - p<0.05 in relation to the indicators of the control group

A feature of the development of doxorubicin CHF, unlike other cardiomyopathies, is toxic damage to myocardial mitochondria, which makes the mitochondria a source of reactive oxygen species and pro-apoatic proteins, and during the deterioration of energy production (a decrease in ATP), activation of oxidative stress, apoptosis is observed [14]. The above mechanisms, realizing their detrimental effect on the heart, ultimately lead to the development of heart failure. A fundamentally important process is the remodeling of the heart. This concept includes: violation of the structure of the contractile apparatus of cardiomyocytes, their functional asymmetry, changes in intercellular interactions, interstitial fibrosis, despiralization of the course of muscle bundles and changes in the shape of the heart cavities. Confirmation that there was a 5-fold increase in cytosol microscopic control of ST2

protein concentration compared to the intact group. ST2 (Suppression of tumorogenicity 2, Growth Stimulation expressed gene 2, stimulating growth factor expressed by gene 2, aka IL1RL1) is a member of the IL-1 receptor superfamily. ST2 is the IL-33 receptor. ST2 - a marker of fibrosis and remodeling of cardiac tissue, released by cardiomyocytes and fibroblasts [15]. An increase in ST2 concentration indicates heart remodeling and the formation of heart failure after 45 days in animals administered with doxorubicin for 14 days. An increase in NT-proBNP by 3.48 times was also recorded, which indicates myocardial damage. Experimental and clinical studies have shown potential markers of heart damage in CHF are circulating atrial and cerebral types of natriuretic peptides (ANP and BNP, respectively), which are elevated in left ventricular dysfunction and heart failure. Levels of these proteins were significantly elevated in the subgroup of patients administered with doxorubicin, who had developed cardiac dysfunction compared to healthy individuals or patients with normal cardiac function [16,17].

We have found that the modeling of CHF by doxorubicin administration led to a 2.6-fold decrease in eNOS activity in the blood of animals compared to the parameters of the intact group, which is an indirect indicator of endothelial dysfunction in this pathology. It is known that during the development of this model of CHF, doxorubicin binds to the eNOS domain, and with an increase in the concentration of doxorubicin, the enzyme do not generate NO, but superoxide, and further suppression of the enzyme expression will occur. Therefore, inhibition of eNOS has far-reaching consequences in the mechanism of decompensated myocardial dysfunction and endothelial dysfunction in CHF [18]. We found an increase in D-dimer by 27 times, which indicates a violation of fibrinolysis and the risk of thrombosis in this model of CHF. Experimental therapy with Metoprolol, Bisoprolol, Nebevalol, Carvedilol and the new drug Hypertril led to a decrease in myocardial damage with varying degrees of effectiveness. Thus, the introduction of Hypertril to rats with CHF led to a decrease in the blood of the main molecular markers of CHF - ST2 and NT-proBNP to the values of intact animals, which indicated its significant cardioprotective effect. Nebivolol competed with Hypertril in the degree of reduction of these markers and reduced the concentration of ST2 by 75.3%, and NT-proBNP by 57.7%. The introduction of Bisoprolol led to a decrease in ST2 and NT-proBNP by 68.4% and 50.3%, respectively. Metoprolol reduced the blood concentration of ST2 and NT-proBNP by 40% and 32%, respectively, Carvedilol provided a decrease in these markers by 41.7% and 35.5%, respectively. Administration of the studied preparations to rats with CHF led to a decrease in D-dimer to varying degrees. However, only in the group of animals treated with a course of Hypertril, this indicator was established at the level of intact animals. We have established an interesting fact of the effect of the studied preparations on the activity of eNOS in the blood of animals with CHF. Thus, the course use of Hypertril leads to a significant increase in eNOS activity - 8.2 times compared with the control group and 3.2 times compared with the intact group, which may indicate the NO-mimetic effect of the drug and its possible endothelioprotective and vasodilation action. Nebivolol also had a positive effect on the activity of eNOS in the blood of animals with CHF - an increase in activity by 6.7 times compared with the control group and 2.5 times compared with the intact group. The introduction of Bisoprolol and Carvedilol had a significant, but much less pronounced effect on eNOS activity in CHF, while Metoprolol did not affect this indicator. The results obtained are in line with our previous studies of cardioprotective effects in various models of heart injury. It is known that Hypertril lowers blood pressure in SHR rats, increases NO production in mitochondria and myocardial cytosol during an increase in eNOS activity and expression [19]. The positive effect of Hypertril on the NO system was accompanied by inhibition of the so-called "parasitic" reactions and their products - cytotoxic derivatives of NO and reduced expression of iNOS [20]. Hypertril reduces the manifestations of mitochondrial dysfunction, which, being an integral aspect of arterial hypertension, contributes to its aggravation by triggering a cascade of molecular and biochemical mechanisms of myocardial damage [7-9]. These mechanisms include disturbances in the L-arginine-NOS-NO system, production of mitochondrial iNOS, oxygen radicals, neutralization of the vasorelaxant effect of NO and its transformation into an active participant in nitrosating stress due to a deficiency of reduced intermediates of the thiol-disulfide system [7,9,14]. It is known that in doxorubicin CHF, Hypertril protects the myocardium and positively affects the morphological and functional parameters of cardiomyocytes, inhibits apoptosis [28]. The β- adrenoblockers studied by us in this work (Carvedilol, Bisoprolol, Metoprolol, Nebivolol) are the main means of standard therapy for heart failure [2,3,5,6]. Demonstrated a decrease in mortality and a certain cardioprotection due to a decrease in

biochemical and molecular markers of heart damage after modeling CHF with doxorubicin. Our data are consistent with those of other investigators who have shown that early use of β- adrenoblockers reduces mortality and improves myocardial contractility in doxorubicin-induced CHF [3,5,6,10,11,12]. However, these studies show that Metoprolol and Bisoprolol, β- adrenoblockers that do not have antioxidant and metabolitotropic properties, do not provide significant cardioprotection, like Carvedilol or Nebivolol [22]. Carvedilol (and presumably other β-blockers as well) is known to prevent dissociation of the eNOS dimer [17]. Unlike Metoprolol and Bisoprolol, Carvedilol inhibits cardiomyocyte apoptosis, which plays a leading role in the progression of decompensated myocardial dysfunction. The anti-apoptotic effect is associated with the ability of carvedilol to reduce ROS-dependent expression of Fas and TNF-a. Although the exact mechanism of cardioprotection of these β- adrenoblockers is still poorly understood [12].

World cardiology has accumulated vast experience in the use of β adrenoblockers in MI. Initially, these drugs were used to limit the area of necrosis, but in this respect they were significantly weaker than thrombolytic therapy or intravenous nitrates. All this served as the basis for identifying the use of β- adrenoblockers in acute myocardial ischemia [23]. Their main mechanism of action is selective binding to β-adrenergic receptors of various organs and tissues, leading to competitive and reversible inhibition of β-adrenoergic stimulation. All β-adrenoblockers are classified into several groups depending on lipo- or hydrophilicity, tropism for β_1-, β_2-, α-receptors (selectivity), ability to activate β-adrenergic receptors (internal sympathomimetic action), duration of pharmacological action [24]. At the same time, the difference between the drugs in this group is so great that many generally dispute the existence of a "class-effect" in the treatment of major cardiovascular diseases [25]. This is important because in such a situation the transfer of data obtained for one drug to another drug should not be used. The main effects of β- adrenoblockers on the cardiovascular system include: hypotensive, anti-ischemic, antiarrhythmic effects, achieved mainly by reducing the heart rate; slow conduction and increase the refractory period of the components of the conduction system, cardiac output, myocardial oxygen demand, prolongation of the diastole phase, reduced release and formation of the main components of the renin-angiotensin system [26]. Also described are effects such as inhibition of platelet aggregation, apoptosis of cardiomyocytes, prevention of atherosclerotic plaque rupture, antioxidant properties [27]. Recently, data have appeared on the NO-mimetic effect of the β- adrenoblocker Nebivolol and the vasodilatory effect caused by it, as well as on the absence of a depressant effect on the work of the left ventricle [28]. In this regard, a promising direction is the creation of drugs that combine in their mechanism of action β_1-adrenergic blocking and NO-mimetic effects, which will not only positively influence cardiohemodynamics and exercise cardioprotection, but also show additional properties arising from a positive effect on the myocardial nitroxidergic system in CHF.

Thus, the obtained results demonstrated the undoubted advantage of the new original molecule (Hypertril) over the basic β-adrenergic blockers (Metoprolol, Nebivolol, Carvedilol, Bisoprolol) and experimentally substantiate further in-depth study to create a drug based on it for the treatment of CHF.

AUTHOR CONTRIBUTIONS

Concept: I.B., P.B., O.P., N.B; Design: I.B., P.B., O.P., V.R., A.P. Control: I.B., P.B., O.P., V.R., N.B., A.P.; Sources: I.B., P.B., O.P., V.R., N.B., A.P.; Materials: I.B., P.B., O.P., V.R., N.B.; Data Collection and/or Processing: I.B., P.B., O.P., V.R., N.B.; Analysis and/or Interpretation: I.B., P.B., O.P., V.R., N.B.; Literature Review: I.B., P.B., O.P., A.P.; Manuscript Writing: I.B., P.B.,O.P., V.R., N.B., A.P.; Critical Review: I.B., P.B.,O.P., V.R., N.B., A.P.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

All manipulations were carried out in accordance with the regulation on the use of animals in biomedical experiments (Strasbourg, 1986, amended in 1998). The protocols of experimental studies

and their results were approved by the decision of the Commission on Bioethics of ZSMU (protocol No. 32 of October 26, 2021).

REFERENCES

1. Bozkurt, B., Coats, A.J., Tsutsui, H., Abdelhamid, C.M., Adamopoulos, S., Albert, N., Anker, S.D., Atherton, J., Böhm, M., Butler, J., Drazner, M.H., Felker, G.M., Filippatos, G., Fiuzat, M., Fonarow, G.C., Gomez-Mesa J.E., Heidenreich, P., Imamura, T., Jankowska, E.A., Januzzi, J., Khazanie, P., Kinugawa, K., Lam, C.S.P., Matsue, Y., Metra, M., Ohtani, T., Piepoli, M.F., Ponikowski, P., Rosano G.M.C., Sakata, Y., Seferovic, P., Starling, R.C., Teerlink, J.R., Vardeny, O., Yamamoto, K., Yancy, C., Zhang, J., Zieroth, S. (2021). Universal definition and classification of heart failure: A report of the Heart Failure Society of America, Heart Failure Association of the European Society of Cardiology, Japanese Heart Failure Society and Writing Committee of the Universal Definition of Heart Failure: Endorsed by the Canadian Heart Failure Society, Heart Failure Association of India, Cardiac Society of Australia and New Zealand, and Chinese Heart Failure Association. European Journal of Heart Failure, 23(3), 352-380. [CrossRef]
2. Urbich, M., Globe, G., Pantiri, K., Heisen, M., Bennison, C., Wirtz, H.S., Di Tanna, G.L. (2020). A systematic review of medical costs associated with heart failure in the USA (2014-2020). PharmacoEconomics, 38, 1219-1236. [CrossRef]
3. 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. (2001). Bisoprolol improves survival in rats with heart failure. Journal of Cardiovascular Pharmacology, 38(1), S55-S58. [CrossRef]
4. Konyakhin, A.Yu. (2009). Modern pathogenetic approaches to the correction of myocardial ischemia. Abstract of the dissertation of the doctor of medical sciences, 47.
5. Wenningmann. N., Knapp, M., Ande, A., Vaidya, T.R., Ait-Oudhia, S. (2019). Insights into doxorubicin-induced cardiotoxicity: Molecular mechanisms, preventive strategies, and early monitoring. Molecular Pharmacology, 96(2), 219-232. [CrossRef]
6. Bien, S., Riad, A., Ritter, C.A., Gratz, M., Olshausen, F., Westermann, D., Kroemer, H.K. (2007). The endothelin receptor blocker bosentan inhibits doxorubicin-induced cardiomyopathy. Cancer Research, 67(21), 10428-10435. [CrossRef]
7. Mazur, I., Belenichev, I., Kucherenko, L., Bukhtiyarova, N., Puzyrenko, A., Khromylova, O., Gorchakova, N. (2019). Antihypertensive and cardioprotective effects of new compound 1-(β-phenylethyl)-4-amino-1, 2, 4-triazolium bromide (Hypertril). European Journal of Pharmacology, 853, 336-344. [CrossRef]
8. Mazur, I.A., Belenichev, I.F., Kolesnik, Yu.M., Kucherenko, L. (2010). 1-(β-phenylethyl)-4-amino-1,2,4-thiatriazolium (MT) bromide with cardioprotective, anti-ishemic, antihypertensive, antioxidant, protein syntetic and energy-tropic action. Patent 2404974.
9. Chekman, I.S., Belenichev, I.F., Kucherenko, L.I., Mazur, I.A., Nagornaia, E.A., Bukhtiiarova, N.V. (2013). Parniuk NV. NO-dependent mechanisms of cardioprotective activity of mt preparation during course administration to SHR rats. Eksperimental'naia i Klinicheskaia Farmakologiia, 76(8), 24-26.
10. Khloponin, D.P. (2009). Analysis of possible mechanisms of pharmacological reversal of cardiac remodeling in chronic heart failure (Doctoral dissertation). Volgograd State Medical University, Volgograd.
11. Sidorov, A.V. (2013). Neurohumoral aspects in the implementation of prognostic effects of β-andreblockers and ACE in chronic heart failure. Doctoral dissertation. Staraya Kupavna; 412 p.
12. Cosentino, F., Bonetti, S., Rudolf, R., Eto, M., Werner-Felmayer, G., Volpe, M., Lüscher, T.F. (2002). Nitric-oxide-mediated relaxations in salt-induced hypertension: Effect of chronic β1-selective receptor blockade. Journal of Hypertension, 20(3), 421-428. [CrossRef]
13. Chen, Y., Hong, X. (2016). Effects of carvedilol reduce conjunctivitis through changes in inflammation, NGF and VEGF levels in a rat model. Experimental and Therapeutic Medicine, 11(5), 1987-1992. [CrossRef]
14. Kolesnik, Yu.M., Chekman, I.S., Mazur, I.A., Belenichev, I.F., Gorchakova, N.O., Nagorna, O.O. (2014). Mechanisms for the development of endothelial dysfunction and prostate endothelial protectors. Journal of the National Academy of Medical Sciences of Ukraine, 20(3), 289-299.
15. Maisel, A.S., Somma, S.D. (2016). Do we need another heart failure biomarker: Focus on soluble suppression on tumoregenicity 2 (sST2). European Heart Journal, 38(30), 2325-2333. [CrossRef]
16. Hayakawa, H., Komada, Y., Hirayama, M., Hori, H., Ito, M., Sakurai, M. (2001). Plasma levels of natriuretic peptides in relation to doxorubicin-induced cardiotoxicity and cardiac function in children with cancer. Medical and Pediatric Oncology, 37 (1), 4-9. [CrossRef]

17. Octavia, Y., Tocchetti, C.G., Gabrielson, K.L., Janssens, S., Crijns, H.J., Moens A.L. (2012). Doxorubicin-induced cardiomyopathy: From molecular mechanisms to therapeutic strategies. Journal of Molecular and Cellular Cardiology, 52(6), 1213-1225. [CrossRef]
18. Neilan, T.G., Blake, S.L., Ichinose, F., Raher, M.J., Buys, E.S., Jassal, D.S., Furutani, E., Perez-Sanz, T.M., Graveline, A., Janssens, S.P., Picard, M.H., Scherrer-Crosbie, M., Bloch, K.D. (2007). Disruption of nitric oxide synthase 3 protects against the cardiac injury, dysfunction, and mortality induced by doxorubicin. Circulation, 116, 506-514. [CrossRef]
19. Belenichev, I., Gorbachova, S., Pavlov, S., Bukhtiyarova, N., Puzyrenko, A., Brek, O. (2021). Neurochemical status of nitric oxide in the settings of the norm, ishemic event of central nervous system, and pharmacological intervention. Georgian Medical New, 315, 169-176.
20. Trujillo. M., Naviliat, M., Alvarez, M.N., Peluffo, G., Radi, R. (2000). Peroxynitrite biochemistry: formation, reactions and detection. Analusis, 28(6), 518-527. [CrossRef]
21. Gilleron, M., Marechal, X., Montaigne, D., Franczak, J., Neviere, R., Lancel, S. (2009). NADPH oxidases participate to doxorubicin-induced cardiac myocyte apoptosis. Biochemical and Biophysical Research Communications, 388(4), 727-731. [CrossRef]
22. Nicol, M., Sadoune, M., Polidano, E., Launay, J.M., Samuel, J.L., Azibani, F. (2021). Doxorubicin-induced and trastuzumab-induced cardiotoxicity in mice is not prevented by metoprolol ESC Heart Failure, Published online in Wiley Online Library, 8(2), 928-937. [CrossRef]
23. Liu, B., Li, H., Qu, H., Sun, B. (2006). Nitric oxide synthase expressions in ADR-induced cardiomyopathy in rats. BMB Reports, 39(6), 759-765. [CrossRef]
24. Belenichev, I.F., Bak, P.G., Abramov, A.V., Kucherenko, L.I., Bukhtiyarova, N.V., Rizhenko, V.P. (2021). ECG analysis in the simulation of chronic heart failure in rats and course administration of a new potential drug “Hypertril”. Pharmacology and Toxicology, 15(1), 20-30.
25. Tassigny, A., Berdeaux, A., Souktani, R., Henry, P., Ghaleb, B. (2008). The volume-sensitive chloride channel inhibitors prevent both contractile dysfunction and apoptosis induced by doxorubicin through PI3kinase, Akt and Erk 1/2. European Journal of Heart Failure, 10(1), 39-46. [CrossRef]
26. Ryzhov, O.A., Ryzhenko, V.P., Levich, S.V., Belenichev, I.F. (2017). Analysis of influence of quantum chemical descriptors on NO-scavenger properties among xanthine derivatives, Biological Markers and Guided Therapy, 4(1), 39-48. [CrossRef]
27. Youn, H.J., Kim, H.S., Jeon, M.H., Lee, J.H., Seo, Y.J., Lee, Y.J., Lee, J.H. (2005). Induction of caspase-independent apoptosis in H9c2 cardiomyocytes by adriamycin treatment. Molecular and Cellular Biochemistry, 270(1), 13-19. [CrossRef]
28. Bak, P.G., Belenichev, I.F., Kucherenko, L.I., Abramov, A.V., Khromylova, O.V. (2021). 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, 68(4), 919-925. [CrossRef]