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Cardioprotective effect of hydroxybenzoic acid ester containing neuroactive acid, compound F-26, in conditions of isoproterenol heart failure in rats

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Abstract

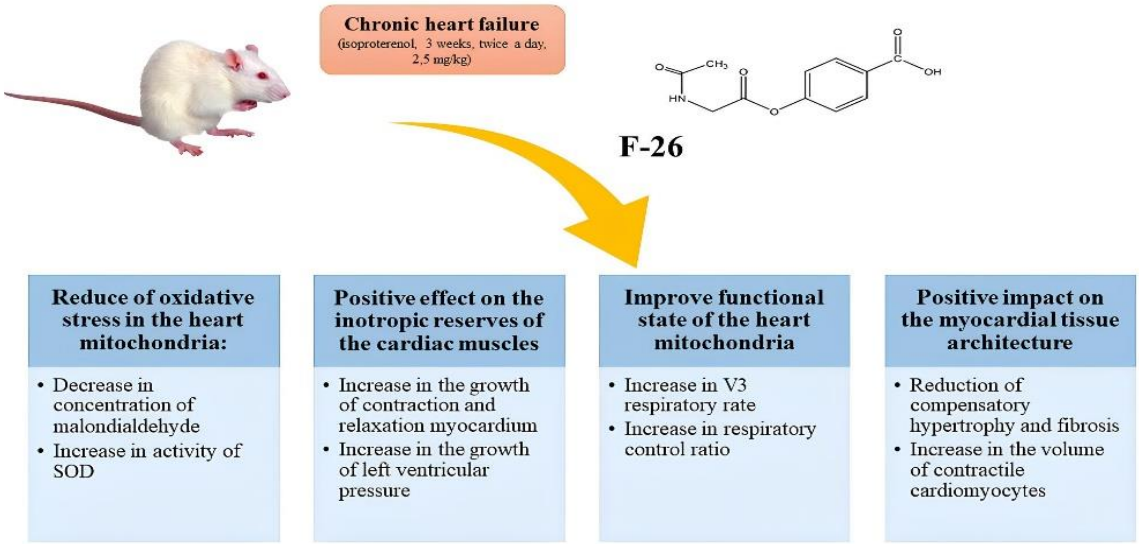
Introduction: Chronic heart failure (CHF) is a leading public health problem. Its most common manifestation is decreased cardiac inotropic reserve. Mitochondrial dysfunction and oxidative stress contribute to the progression of CHF. Hydroxybenzoic acid derivatives limit mitochondrial dysfunction and oxidative stress, thereby reducing their damaging effects on the heart.

Materials and Methods: Chronic heart failure (CHF) was modeled by intraperitoneal administration of L-isoproterenol to male Wistar rats at a dose of 2.5 mg twice daily for 21 days. Four groups of 14 animals each were formed: intact animals, a control group with CHF that received saline solution, and experimental animals with CHF that received compound F-26 at a dose of 7.9 mg/kg and mildronate –50 mg/kg intraperitoneally once daily throughout the CHF simulation period. Cardiac inotropic function was assessed using functional tests: volume loading, adrenergic receptor activation, and occlusion of the ascending aorta. Mitochondrial oxygen uptake was determined using polarography. Myocardial morphometric analysis was performed using micrographs.

Results and Discussion: Compound F-26 has a positive effect on cardiac inotropic reserves, as evidenced by increases in myocardial contraction and relaxation rates, left ventricular pressure, and maximum structural activity intensity (MSI) during functional tests. Compound F-26 improves mitochondrial respiration, reduces MDA concentration, increases SOD activity, and has a positive effect on the cross-sectional area, thickness, and volume fraction of cardiomyocytes and interstitium.

Conclusion: Compound F-26 improves cardiac inotropic function, mitochondrial function, the antioxidant system of cardiomyocytes, and morphometric parameters.

Graphical Abstract



Keywords

chronic heart failure, mitochondrial dysfunction, oxidative stress, hydroxybenzoic acid derivatives, morphometric parameters of cardiomyocytes, compound F-26, mildronate

Introduction

Chronic heart failure (CHF) is currently one of the leading healthcare problems, burdening the healthcare system with high financial costs due to frequent hospitalizations, complications, comorbidities, and a poor prognosis (Savarese et al. 2023). CHF is defined as a clinical syndrome characterized by symptoms of cardiac damage, elevated natriuretic peptide levels, and congestion in the systemic or pulmonary blood flow (Bozkurt et al. 2021). The most common manifestation of CHF is decreased inotropic reserves of the heart muscle, which eventually leads to decompensation and damage to internal organs (Larina et al. 2023). The pathogenesis of CHF is largely associated with mitochondrial dysfunction, which plays a key role in the synthesis of ATP energy to maintain cardiac contractility. Furthermore, mitochondrial dysfunction is accompanied by increased generation of reactive oxygen species (ROS), depletion of the antioxidant system, and the development of oxidative stress. Oxidative stress increases Ca2+ influx into cardiomyocytes, which also negatively impacts myocardial contractility, impairs cardiomyocyte relaxation, and increases left ventricular filling pressure. It can induce increased production of proinflammatory cytokines and fibroblast activation in the extracellular matrix, leading to interstitial fibrosis and passive myocardial stiffness (Bhullar et al. 2023). Thus, oxidative stress acts as a potent damaging factor, aggravating mitochondrial dysfunction, causing cardiomyocyte apoptosis, initiating fibrosis, and promoting pathological cardiac remodeling. As a result, a vicious cycle is formed, in which mitochondrial dysfunction, oxidative stress, and heart failure are inextricably linked and mutually aggravate one another.

Therefore, mitochondria may be a potential target for the treatment of heart failure (Demko et al. 2022; Hinton et al. 2024).

Literature data indicate that a number of hydroxybenzoic acid derivatives and neuroactive acids have the ability to limit mitochondrial dysfunction and oxidative stress, reducing the damaging effects of various factors on the heart muscle (Denisyuk et al. 2016; Li et al. 2021). Due to the phenolic group present in hydroxybenzoic acid and its derivatives, they are able to react with various types of free radicals (hydroxyl •OH or peroxyl radicals ROO•), forming stable molecular complexes. During neutralization, hydroxybenzoic acid and its derivatives donate a hydrogen atom or electron to the free radical, converting it into inactive products and preventing oxidation chain reactions that can damage cells and tissues. However, it's not just their ability to scavenge oxidants that makes hydroxybenzoic acid derivatives a promising target for the treatment of CHF. Another defining property is their ability to modify cellular signaling

processes, leading to a multiplicative effect. One example is the activation of the Nrf2 (Nuclear factor erythroid 2-related factor 2) pathway, a transcription factor that regulates the expression of genes encoding antioxidant enzymes. This leads to the enhancement of multiple endogenous antioxidant mechanisms, as the activity of this signaling pathway is reduced during oxidative stress.

All of this creates the prerequisites for studying the effects of hydroxybenzoic acid derivatives, such as the compound coded F-26, on the development of CHF. The compound contains the neuroactive acid glycine, which also exhibits antioxidant activity, as demonstrated in experimental myocardial infarction in rabbits; in cerebral cortex sections of rats under anoxia; in hypoxia caused by cessation of normoxic perfusion; in isolated rat hearts; and in cultures of ischemic rat cardiomyocytes (Ruiz-Meana et al. 2004; Rodriguez-Sinovas et al. 2005; Tonshin et al. 2007).

Therefore, **the aim of our study** was to investigate the cardioprotective effect of compound F-26, a hydroxybenzoic acid derivative containing the neuroactive acid.

Materials and methods

Experimental animals

The experiments were conducted on male Wistar rats aged 4 months, n=56. Their average weight was 260–280 g. The animals were housed under standard vivarium conditions in accordance with the guidelines of The European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (The European Convention, 1986) and The European Union Directive (EU Directive 2010/63/EU). They had free access to water and food and 12 hours of daylight. The protocol of the experimental study was reviewed by the Regional Independent Ethics Committee (Local Ethics Committee, Volgograd, Russia, minutes No. 2021/064-DI 22.09. 2021).

Drugs and treatment

The drugs under study – compound F-26 (sodium salt of N-acetyl glycine ester and 4-hydroxybenzoic acid) (Fig. 1) was synthesized at the Department of Chemistry, Volgograd State Medical University (Volgograd, Russia); mildronate, its comparator agent, (active substance is meldonium) was used as a ready-for-use solution for injections (Grindex, Latvia). Saline solution was applied as a solvent for the studied agents. The investigated compound was introduced intraperitoneally at a dose of 7.9 mg/kg, mildronate – 50 mg/kg).

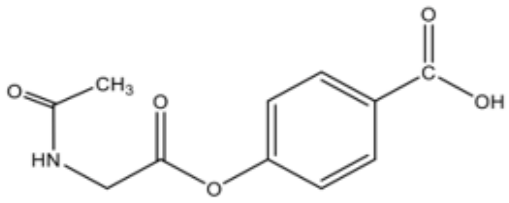


Figure 1. Chemical structure of compound F-26.

CHF modeling

CHF was modeled by intraperitoneal injection of L-isoproterenol (SigmaAldrich, USA) at a dose of 2.5 mg/kg twice a day for 21 days (Ennis et al., 2003).

Experimental design

Before the experiment, the following groups were formed: 1) intact (n=14), which were administered saline; 2) control – CHF + saline (n=14), animals that received L-isoproterenol and saline; 3) experimental – CHF + F-26 (n=14), animals that were administered L-isoproterenol and compound F-26; 4) experimental – CHF + mildronate (n=14), rats that were administered L-isoproterenol and mildronate. Saline, the test compound and mildronate were administered intraperitoneally, once a day for 21 days.

Assessment of myocardial contractility

Twenty-one days after the start of the experiment, the animals were narcotized (chloral hydrate, 350 mg/kg) and preoperative preparation was performed. After that, access was provided to the jugular vein for drug administration, to the trachea for tracheostomy and connection to an

artificial lung ventilation (ALV) apparatus (RWD R415 VentStar, USA). A catheter connected to a pressure sensor (Biopac Systems, USA) was introduced through the heart apex to the left ventricle to register the following indicators: myocardial contraction rate (+dP/dtmax, mmHg/sec), myocardial relaxation rate (-dP/dtmax, mmHg/sec), left ventricular pressure (LVP), and heart rate (HR) (beats per minute). After the period of stabilization (10 min) and registration of baseline indicators, load tests were conducted: volume load test (intravenous bolus administration of 0.9% NaCl solution at a rate of 0.3 mg per 100g of the animal weight), adrenoreactivity test (IV introduction of adrenaline at a dilution of 10^{-7}g/L at a dose of 0.1 mL per 100g of the animal weight) and isometric load test (occlusion of the ascending aorta for 30 seconds). Maximum intensity of structural performance (MISP) was calculated as shown below, formula 1 (Mironov 2012):

$$\mathrm{X\ (mmHg/mg * min)} = \frac{(\mathrm{LVPavg} \times \mathrm{HRavg})}{(\text{left ventricular mass} + 13 \text{ of the interventricular septum})} \quad (1)$$

Isolation of mitochondria

After the load tests had been performed, the heart was washed in ice-cold saline solution and homogenized at a temperature of 4 °C in a Potter-Elvehjem homogenizer in an isolation medium containing 220mM mannite, 100mM sucrose, 1mM EDTA, 4mM KH2PO4, 20мM HEPES, pH=7.3. Mitochondria were obtained using standard differential centrifugation (Lanza and Nair 2009). The homogenates were centrifuged at 600 G for 10 minutes to sediment debris and intact cells. The supernatant was centrifuged for 20 minutes (8000 G). The sediment was resuspended and used as a mitochondrial fraction, in which respiration intensity, the concentration of lipid peroxidation products and antioxidant enzyme activities were determined.

Assessment of mitochondrial function

Mitochondrial oxygen consumption rate was determined by means of polarography using an Oxytherm System polarograph with the Clark electrode (Hansatech Instruments, UK). The functional state of mitochondria was investigated based on the protocol described by Lanza and Nair (2009). Previously, all the solutions (except ADP) were thermostated for 20 minutes at 33 °C. The reagents required to make solutions were obtained from Sigma-Aldrich (USA).To explore the metabolic states according to Chance, 100 μL of the following substances were consecutively introduced into a 1mL thermostated polarographic cell with the polarographic medium (0.5 mM EDTA, mM $MgCl_2$ *$6H_2O$, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 110 mM sucrose, 1 g/L BSA, pH=7.4): V3(I) (oxygen consumption rate in the presents oxidation substrate of complex I and ADP, oxidation substrates of complex I), namely, in the state of oxidative phosporylation involving the activation of Complex I – ADP (200μM); V3(II) – rotenone, an inhibitor of complex I (0.5 μM), and ADP; V4 – oligomycin, an inhibitor of ATP-synthase (2.5 mM); V(uncoupling)–carbonyl-cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP), a proton ionophore (0.05 mM). The rate of oxygen consumption was expressed in nmol O_2 /min/mg protein. To assess the coupling of respiration and phosphorylation, we calculated the respiratory control ratio (RCR) (V3/V4 ratio) (Brand and Nicholls 2011; Popova et al. 2021).

Determination of the level of lipid product oxidation and antioxidant enzyme activity

The aliquots of mitochondrial suspension were exposed to a single freeze-thaw cycle for mitochondria to be disrupted. The concentration of malondialdehyde (MDA) was determined, after which the activity of antioxidant enzymes superoxide dismutase (SOD) was assessed.

The method of determining MDA content is based on measuring the amount of TBC-reactive products, which are formed when 0.7% thiobarbituric acid solution is boiled with isolated mitochondria in acidic medium (with 1.3% H_3PO_4 added). We estimated the optical density of the obtained solution at 532 nm using a Helios γ spectrophotometer (Thermo Electron Corporation, UK). The MDA level was expressed in μmol MDA/mg protein (Stalnaya et al. 1977).

The total activity of superoxide dismutase was determined by the degree of inhibition of the oxidation reaction of quercetin (Kostyuk et al. 1990). 100 μL of a solution of quercetin in dimethyl sulfoxide (DMSO) (0.2 mg/mL) and 150 μL of the mitochondrial suspension were added to 3.4 mL of phosphate buffer (pH 7.8) containing 0.08 mM ethylenediaminetetraacetate and 0.8 mM tetramethylethylenediamine. The initial optical density of the samples and after 20 minutes at λ = 406 nm were measured. The percentage of inhibition was calculated using the formula 2:

$$\mathrm{I} = 100 - \left(\frac{\mathrm{As0'} - \mathrm{As20'}}{\mathrm{Ac0'} - \mathrm{Ac20'}}\right) \times 100, \quad (2)$$

where: I is the percentage of inhibition; As0' and As20' are the initial optical densities of the test sample and after 20 minutes of incubation, respectively; Ac0' and Acontr20' are the initial optical densities of the control sample and after 20 minutes of incubation, respectively. SOD activity was expressed in arbitrary units per 1 mg of protein.

Protein concentrations in the samples were assessed using a Pierce BCA Protein Assay Kit (Thermo Scientific, USA).

Histological examination

For histological examination, the hearts of some animals were removed, and fragments of the left ventricular myocardium measuring 0.5 x 0.5 cm were cut out. After fixing the biomaterial for 24 hours in 10% buffered neutral formalin, dehydration was carried out in a series of alcohols of increasing concentration, followed by treatment in xylene and embedding in histomyx (BioVitrum, Russia). Using a rotary microtome (Thermofisher HM340E, USA), 6.0 µm thick sections were obtained, which were mounted on glass slides (Thermo scientific, Polysine slides, USA). The resulting micropreparations were stained with hematoxylin (NPF Abris+, Russia) and eosin (OOO Labiko, Russia) according to the standard method (Mondal 2019). Morphological changes in the myocardium were assessed by the presence of areas of fibrosis, atrophy and/or hypertrophy and wave-like deformation of cardiomyocytes, foci of their decay, lymphohistiocytic infiltration, microhemorrhages, stasis and sludge in the vessels, uneven staining of cardiomyocytes and their nuclei. Morphometric study of the myocardium was performed using microphotographs processed using MCview software (LOMO-microsystems, Russia). We calculated the volume fractions of cardiomyocytes, vessels and interstitium per unit area of the section, and also measured the cross-sectional area of cardiomyocytes at the level of the nuclei, the thickness of cardiomyocytes in the widest part and the area of their nuclei in the longitudinal section in 10 random fields of view of each section (Mondal 2019).

Statistical analysis

Statistical processing was performed using the GraphPad Prism 9.5 program. The normality of distribution was determined by the Shapiro-Wilk test. For parametric paired tests, Student's t-test was used, for multiple comparisons – the Brown-Forsythe and Welch test, for nonparametric tests – the Mann-Whitney and Kruskal-Wallis tests with Dunn's post-test. The results are presented as (M ± SE), where M is the mean value, SE is the standard error of the mean. The differences were assumed statistically significant at $p<0.05$.

Results

Effect of the studied compound on rats' cardiac contractility

Increasing the preload in the control group resulted in the growth of +dP/dt max, -dP/dt max, and LVP by 17.7; 12.9 and 14.0%, respectively, relative to the initial data, which was 3.8; 3.9 and 2.7 ($p < 0.05$) times lower compared to that in the intact group, where the increase was 68.0%, 50.7% and 38.3%, respectively. In rats receiving compound F-26, the increase in +dP/dt max was 30.8%, in -dP/dt max – 33.2%, and in LVP – 31.1%, which was 1.7; 2.6, 2.2 ($p < 0.05$) times higher compared to that in the control group. The studied parameters in rats receiving mildronate were 1.9; 1.3, 2.2 times higher than in the control group and amounted to 34.4%, 16.9% and 30.6% ($p<0.05$), respectively (Table 1)

Table 1. Effect of compound F-26 and mildronate on the rate of contraction, relaxation of the myocardium, and LVP in Wistar rats (n=40) under volume loading in conditions of isoproterenol heart failure (M± SE)

Animal groups	Myocardial contraction rate (+dP/dt max, mmHg/s)	Myocardial relaxation rate (-dP/dt max, mmHg/s)	Left ventricular pressure (LVP, mmHg)
Intact (n=10)	9706.6± 964.3 (68.0%)	6166.8± 448.3 (50.7%)	160.3±4.0 (38.3%)
CHF + saline solution (n=10)	3078.3±393.6* (17.7%)	2321.6±211.6* (12.9%)	109.0±5.0* (14.0%)
CHF +F-26 (n=9)	5613.0±476.9# (30.8%)	4300.8±399.8# (33.2%)	139.0±3.3# (31.1%)
CHF+mildronate (n=11)	5336.3±304.1# (34.4%)	3421.9±200.8# (16.9%)	127.5±4.5# (30.6%)

Note: * – $p<0.05$ compared to the intact group; # – $p<0.05$ compared to the control group; in brackets –% increase; CHF – chronic heart failure; LVP – left ventricular pressure; +dP/dt – myocardial contraction rate; -dP/dt – myocardial relaxation rate.

Stimulation of adrenoreceptors caused an increase in the rates of contraction and relaxation of the LVP in the control group by 22.2; 19.1 and 18.2%, respectively, which was 3.5, 4.1 and 3 ($p<0.05$) times lower compared to those in the intact group. In the CHF+F-26 group, the increase in the rate of myocardial contraction was 46.0% – 2 times, the relaxation rate was 36.4% – 1.9 times, and the LVP was 37.8% – 2.1 ($p<0.05$) times higher compared to those in the control group. In rats with CHF, which were administered mildronate, the increases in +dP/dt max, -dP/dt max were 43.4 and 35.51%, which was 2.0 and 1.9 ($p<0.05$) times higher than in the control group. The increase in LVP was 33.2%, which was 1.8 times higher than in the control group (Table 2).

Table 2. Effect of compound F-26 and mildronate on the rates of contraction, relaxation of the myocardium, and LVP in Wistar rats (n=40) during stimulation of cardiac adrenoreceptors in conditions of isoproterenol heart failure (M± SE)

Animal groups	Myocardial contraction rate (+dP/dt max, mmHg/s)	Myocardial relaxation rate (-dP/dt max, mmHg/s)	Left ventricular pressure (LVP, mmHg)
Intact (n=10)	11057.6±424.7 (77.2%)	6847.4± 517.5 (79.1%)	180.0±6.7 (54.5%)
CHF + saline solution (n=10)	3285.4± 306.8* (22.2%)	3180.5± 240.9* (19.1%)	117.4±2.4* (18.2%)
CHF +F-26 (n=9)	5381.6±927.1# (46.0%)	4060.4±450.3# (36.4%)	143.4±8.6# (37.8%)
CHF+mildronate (n=11)	5418.4± 712.3# (43.4%)	3537.9± 353.7# (35.5%)	143.3±6.5 (33.2%)

Note: * – $p<0.05$ compared to the intact group; # – $p<0.05$ compared to the control group; in brackets – % increase; CHF – chronic heart failure; LVP – left ventricular pressure; +dP/dt – myocardial contraction rate; - dP/dt – myocardial relaxation rate.

Under conditions of the increased afterload for 5 sec of ascending aortic arch occlusion, the increases in +dP/dt max, -dP/dt max, and LVP in the control group were 10; 10 and 3 ($p<0.05$) times less than in the intact group and amounted to 11.2; 10.8 and 24.0%, respectively. The following values were obtained for 5 sec in the experimental groups: the increase in the contraction velocity in the CHF+F-26 group was 55.6% relative to the initial data and 5 times higher than in the control group, and in animals with CHF receiving mildronate – 75.9%, 6.7 ($p<0.05$) times higher than in the control. In animals with CHF that had been administered compound F-26, the increase in -dP/dt max was 66.0% in relation to the initial values, which is 6.1 ($p<0.05$) times higher than in the control group; in the group of rats that had received mildronate, the increase was 53.5%, 5.0 times higher than in the control group. The increase in LVP for 5 sec in the CHF+F26 and CHF+mildronate groups was 61.1% and 73.6%, respectively, in comparison with the initial data, and 2.5 and 3 ($p<0.05$) times higher, respectively, than in the control group (Table 3).

Table 3. Effect of compound F-26 and mildronate on the rates of contraction, relaxation of the myocardium and LVP in Wistar rats (n=40) at 5 seconds of isometric exercise in conditions of isoproterenol heart failure (M± SE)

Animal groups	Myocardial contraction rate (+dP/dt max, mmHg/s)	Myocardial relaxation rate (-dP/dt max, mmHg/s)	Left ventricular pressure (LVP, mmHg)
Intact (n=10)	12325.0± 396.9 (121.8%)	6798.6± 376.7 (108.5%)	201.8± 1.8 (72.3%)
CHF + saline solution (n=10)	3915.4± 474.8* (11.2%)	2867.3± 284.4* (10.8%)	116.5±5.7* (24.0%)
CHF +F-26 (n=9)	5831.6± 491.0# (55.6%)	4411.4±250.0# (66.0%)	179.5±7.2# (61.1%)
CHF+mildronate (n=11)	5797.3± 435.8# (75.9%)	3974.3± 336.8# (53.5%)	174.5±6.8 (73.6%)

Note: * – $p<0.05$ compared to the intact group; # – $p<0.05$ compared to the control group; in brackets –% increase; CHF – chronic heart failure; LVP – left ventricular pressure; +dP/dt – myocardial contraction rate; - dP/dt – myocardial relaxation rate.

At 30 sec in response to the increase in afterload, the studied parameters in the control group were 12; 13 and 3 ($p<0.05$) times lower than in intact animals and amounted to 8.4%, 7.7%, 20.4%, respectively, in relation to the initial data. In animals receiving compound F-26, the increases in +dP/dt max, -dP/dt max, and LVP were 49.8%, 65.1%, 56.6%, which is 5.9; 8.4 and 2.8 ($p<0.05$) times higher compared to those in the control group. The increases in the studied parameters in the CHF+mildronate group at 30 seconds of the experiment were 71.5%, 56.2%,

68.8%, which is 8.5 ($p<0.05$), 7.3 ($p=0.087$), and 3.4 ($p<0.05$) times higher compared to those in the control group (Table 4).

The increase in MISP in the control group at 5 and 30 seconds was 5.8 and 7.6 ($p < 0.05$) times, respectively, compared to that in intact animals. In the CHF + F-26 group, MISP was 118.5 and 115.3 mm Hg/mg * min at 5 and 50 seconds of occlusion, respectively, which was 2.2 ($p < 0.05$) times higher than in the control group in both cases. The increase in the indicator in the group of animals receiving mildronate, at 5 sec MISP was 105.4 mmHg / mg * min, which was 2 ($p < 0.05$) times higher than in the control group, at 30 seconds – 102.1 mmHg/mg*min, which was also 2 ($p<0.05$) times higher (Figs 2-3).

Table 4. Effect of compound F-26 and mildronate on the increase in the rates of contraction, relaxation of the myocardium and LVP in Wistar rats (n=40) at 30 s of isometric exercise in conditions of isoproterenol heart failure (M± SE).

Animal groups	Myocardial contraction rate (+dP/dt max, mmHg/s)	Myocardial relaxation rate (-dP/dt max, mmHg/s)	Left ventricular pressure (LVP, mmHg)
Intact (n=10)	11057.6±424.7 (104.4%)	6517.1±359.6 (99.3%)	195.8±4.6 (67.1%)
CHF + saline solution (n=10)	3285.4±306.8* (8.4%)	3180.5±240.9* (7.7%)	117.4±2.4* (20.4%)
CHF +F-26 (n=9)	5381.9±927.1# (49.8%)	4060.5±450.3# (65.1%)	143.4±8.6 (56.6%)
CHF+mildronate (n=11)	5418.38±712.3# (71.5%)	3537.9±353.7# (56.2%)	143.2±6.5# (68.8%)

Note: * – $p<0.05$ compared to the intact group; # – $p<0.05$ compared to the control group; in brackets – % increase; CHF – chronic heart failure; LVP – left ventricular pressure; +dP/dt – myocardial contraction rate; - dP/dt – myocardial relaxation rate.

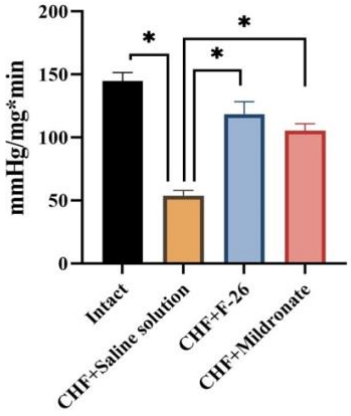


Figure 2. MISP at 5 s of isometric load in CHF conditions in Wistar rats (n=40). *Note:* * – $p<0.05$; CHF – chronic heart failure; MISP – maximum intensity of structural performance.

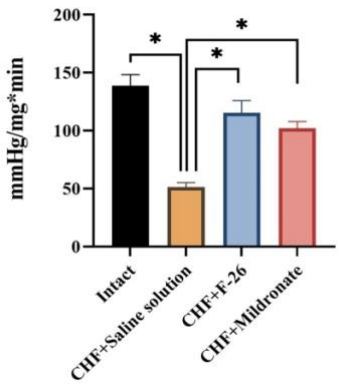


Figure 3. MISP at 30 s of isometric load in CHF conditions in Wistar rats (n=40). *Note:* * – $p<0.05$; CHF – chronic heart failure; MISP – maximum intensity of structural performance.

Effect of the studied compound on the functional activity of rats' cardiac mitochondria

It was also revealed that CHF causes statistically significant changes ($p<0.05$) in the V4 respiratory rate (13.1 in the control group versus 10.9 in the intact group). Compound F-26 and mildronate did not cause statistically significant changes in this indicator, in these groups it was 11.0 and 11.3, respectively). Under conditions of stimulated respiration, the oxygen absorption rate during the work of complex I of the electron transport chain in rats of the control group was 20.7 versus 45.5 in the intact group, which was 2.2 ($p<0.05$) times lower. In rats receiving compound F-26, this indicator was 31.9, mildronate – 30.0, ($p<0.05$), which is statistically significantly higher in relation to the control. During the work of complex II in rats of the control group, the oxygen uptake rate was also statistically significantly lower than in the intact group (24.6 versus 51.6, $p<0.05$). Compound F-26 and mildronate had a positive effect on the work of complex II, which is confirmed by a statistically significant difference in the oxygen uptake rate compared to those in the control group (Fig. 4). Against the background of CHF, the respiratory control coefficients of complexes I and II statistically significantly differed from those in the intact group: RCC of complex I – 1.6 versus 4.3 ($p<0.05$), respectively, RCC of complex II – 1.9 versus 4.9 ($p<0.05$). The test compound F-26 and the comparison drug mildronate limit the damaging effect of CHF on mitochondria, as evidenced by higher values of the RC I complex: 2.9 and 2.7, respectively ($p<0.05$), and the RC II complex: 3.6 and 3.3 ($p<0.05$), respectively (Fig. 5).

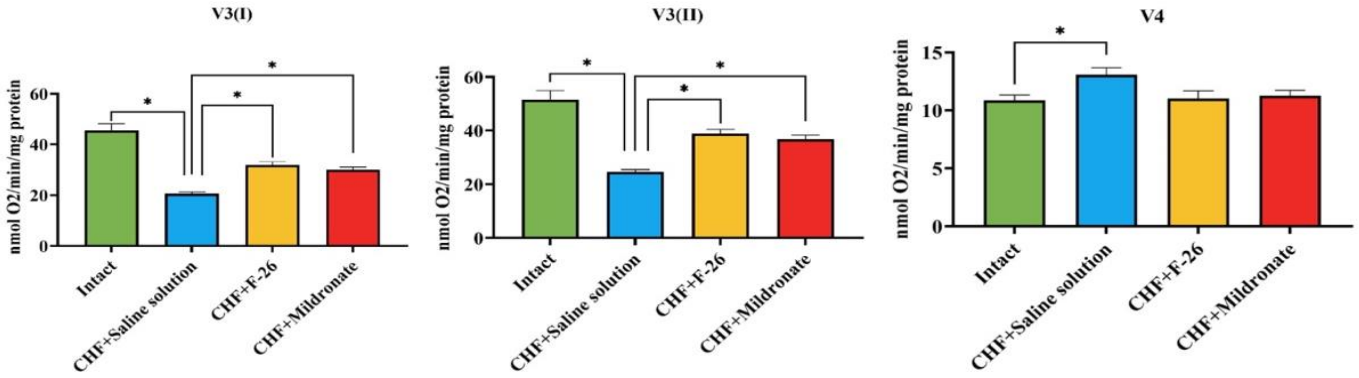


Figure 4. Effect of compound F-26 and mildronate on the functional state of intact Wistar rats heart mitochondria and under experimental CHF conditions (n=43). *Note:* * – $p<0.05$.

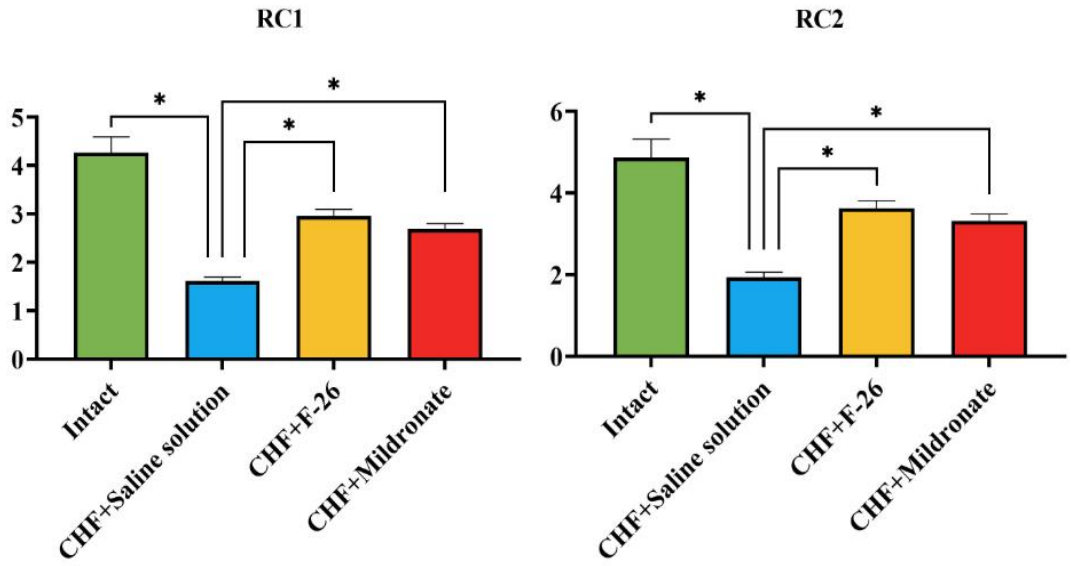


Figure 5. Changes in the respiratory control (RC) coefficient of in intact Wistar rats heart mitochondria and under experimental CHF conditions (n=43). *Note:* * – $p<0.05$.

Effect of the studied compound on MDA and the activity of SOD of cardiac mitochondria in rats

In the control group animals, the MDA concentration was 6.5 times higher ($p<0.05$), and the total SOD activity was 1.5 times lower ($p<0.05$) compared to those in intact animals (Fig. 6). Compound F-26 and mildronate reduced the MDA concentration by 2.1 times and 1.7 times and contributed to an increase in SOD activity by 2.0 and 2.1 times, respectively, compared to those in the control group.

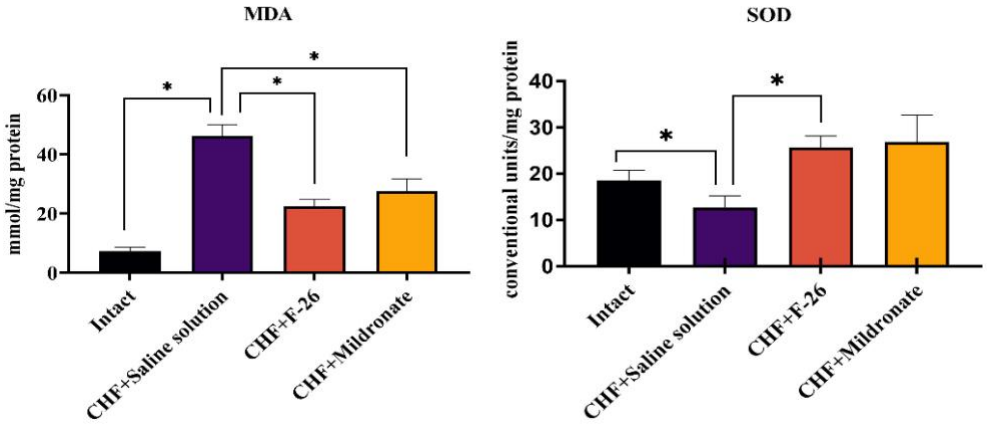


Figure 6. Effect of compound F-26 and mildronate on MDA concentration and SOD activity of cardiac mitochondria in Wistar rats intact and with CHF (n=43). *Note:* * $p<0.05$

Histological examination

Microscopic examination of the myocardium of intact animals revealed that cardiomyocytes were of relatively uniform thickness with well-defined transverse striations (Fig. 7 A). In rats with CHF, pronounced hypertrophy of cardiomyocytes was observed; areas of myofibrils with fragmentation and absence of transverse striations were determined, while the nuclei were polymorphic and slightly enlarged. The microscopic picture was also characterized by foci of interstitial fibrosis and lymphohistiocytic infiltration (Fig. 7 B). The morphometric analysis confirmed morphological changes in the myocardium – in animals with CHF, the cross-sectional area of cardiomyocytes and their thickness were statistically significantly higher by 48.7% and 25.8%, respectively, compared to those in intact rats. A decrease in the proportion of contractile cardiomyocytes and an increase in the volume fraction of the interstitium by 20.4% indicated the proliferation of connective tissue, and probably perivascular and intermuscular edema (Table 5).

In rats with CHF that had received compound F-26, less pronounced structural changes in the myocardium were observed: moderate hypertrophy of individual muscle fibers, cardiomyocytes without wave-like deformation and fragmentation were surrounded by a thin layer of loose fibrous connective tissue with isolated hemorrhages and erythrostasis (Fig. 7C; Table 5). In animals that had received mildronate against the background of CHF, an improvement in the morphological picture of the myocardium was revealed: cardiomyocytes of uniform thickness, slightly hypertrophied with a reduced nuclear size and no fragmentation of muscle fibers (Fig. 7D, Table 5).

Table 5. Effect of compound F-26 and mildronate on morphometric parameters of the left ventricular myocardium in intact Wistar rats and with CHF (n=28)

Animal groups	Cross-sectional area of CMC, μm2	Cardiomyocyte thickness, μm	S of cardiomyocyte nuclei, μm2	Volume fraction of cardiomyocytes, %	Volume fraction of interstitium, %	Volume fraction of vessels, %
Intact	252.1±18.0	14.7±2.0	41.6±4.1	94.1±1.9	4.9±2.2	1.0±0.5
CHF + saline solution	375.0±28.5*	18.5±2.9*	48.9±4.9*	89.9±5.2*	9.2±4.5*	0.9±0.9
CHF+F-26	278.3± 28.7#	15.5 ± 1.9#	46.7 ± 6.2	95.5 ± 1.1#	4.2 ± 1.1#	0.3 ± 0.6
CHF+mildronate	307.2±12.5#	16.1±5.4	33.7±8.9#	93.2±1.6	5.2±1.7	1.6±0.1

Note: * – compared with the intact group; # – compared with the control group, $p<0.05$.

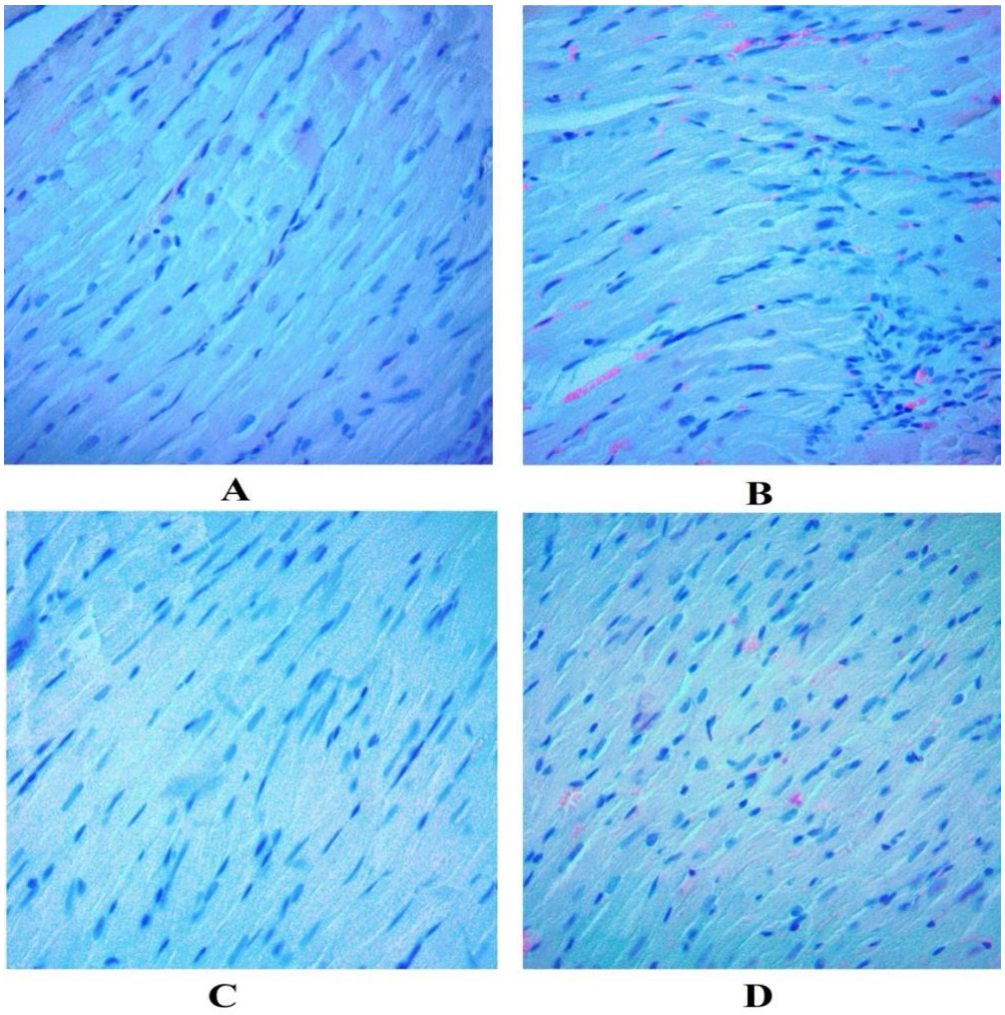


Figure 7. Morphological changes in the myocardium of the left ventricle in animals of the experimental groups (x400, hematoxylin and eosin staining): **A** – intact animals; **B** – CHF + saline group; **C** – CHF + F-26 compound group; D – CHF + mildronate group. Note: CHF – chronic heart failure.

Discussion

Heart failure induced by intraperitoneal administration of isoproterenol at a dose of 2.5 mg 2 times a day for 21 days leads to a marked decrease in cardiac inotropic reserves, as indicated by a decrease in the rate of myocardial contraction and relaxation, as well as LVP and MISP in the control group. According to the literature, hydroxybenzoic acids have a powerful cardioprotective effect by inhibiting smooth muscle matrix invasion in the vascular wall, protecting angiotensin II-induced hypertension in rats, as well as reducing the manifestations of endothelial dysfunction and promoting the formation of nitric oxide (Afnan et al. 2022). Neuroactive acids are also able to reduce the negative impact of various factors on the heart muscle. Thus, taurine in an experiment with aortic occlusion reduced cardiomyocyte hypertrophy and fibrosis, and also weakened the severity of apoptosis and oxidative stress (Liu et al 2020). Gabapentin (a GABA derivative) in a doxorubicin heart failure model improved ECG parameters, heart rate, and reduced the ST segment. At the same time, the level of myocardial damage biomarkers, namely, the activity of creatine kinase, aspartate aminotransferase, and lactate dehydrogenase in the blood serum, decreased (Samra et al. 2021). The data we obtained indicate that compound F-26, which is a derivative of hydroxybenzoic acid containing neuroactive acid in its composition, has a positive effect on the inotropic reserves of the cardiac muscle, as indicated by an increase in the growth rate of contraction and relaxation of the myocardium, as well as the left ventricular contraction rate and the left ventricular contraction rate during exercise tests.

It was found that CHF disrupts the function of mitochondria and the antioxidant system. Compound F-26, a phenolic acid derivative, can neutralize reactive oxygen species. The

neuroactive acid in F-26 can also reduce the negative impact of free radical oxidation processes, as indicated by a decrease in the concentration of MDA, as well as an increase in SOD activity, which is consistent with literature data (Yalameha et al. 2023). Microscopic and morphometric analysis of the myocardium showed that compound F-26 reduces the manifestations of compensatory hypertrophy, which was expressed in a decrease in the cross-sectional area and thickness of cardiomyocytes, which are close in value to the intact group. It was also found that in animals receiving the test compound, the volume fraction of cardiomyocytes increased and the proportion of interstitium decreased, which confirms a decrease in the development of fibrosis under the influence of F-26 and, consequently, the severity of CHF. This is supported by the positive effect of the studied compound on the inotropic function of the heart, revealed during functional tests. Perhaps this effect is due to the presence of neuroactive acid in the compound. Thus, glycine in an experiment with transverse narrowing of the aorta and the introduction of angiotensin II statistically significantly reduced cardiomyocyte hypertrophy and improved the histological structure (Lu et al. 2017).

The drug mildronate and its cardioprotective effect have been widely studied. Under ischemic conditions, it improves the delivery and consumption of oxygen in cardiomyocytes, which reduces cellular energy costs, the formation of free radicals and the development of oxidative stress. The use of this drug improves the contractile function of the heart and the functioning of mitochondria in various pathologies (Statsenko et al. 2021).

Conclusion

Compound F-26, a hydroxybenzoic acid ester containing a neuroactive acid, at a dose of 7.9 mg/kg intraperitoneally once daily for 21 days, exerts a cardioprotective effect in isoproterenol-induced heart failure in rats, comparable to the reference drug mildronate, as evidenced by an increase in the growth rate of myocardial contraction and relaxation, LVP, and MIFP during exercise tests in animals with CHF compared to those the control group. The cardioprotective effect of the compound is likely due to improved mitochondrial respiratory function, increased mitochondrial oxygen consumption, and the respiratory control coefficient, which is likely achieved by reducing MDA concentrations and increasing SOD activity in cardiomyocytes, as well as limiting changes in the morphometric parameters of cardiac cells caused by heart failure.

Additional Information

Conflict of interest

The authors declare the absence of a conflict of interests.

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The authors have no funding to report.

Ethics statement

The protocol of the experimental study was reviewed by the Regional Independent Ethics Committee (Local Ethics Committee, minutes No. 2021/064-DI 22.09. 2021).

Data availability

All of the data that support the findings of this study are available in the main text.

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