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ABSTRACT

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MODULATORY EFFECTS OF PORCINE CARDIAC CRYOEXTRACT ON GLYCOGENOLYSIS IN EXPERIMENTAL MODELS OF MYOCARDIAL DYSTROPHY

Background. Myocardiodystrophy represents a severe metabolic disturbance in the cardiac muscle, leading to structural and functional alterations in cardiomyocytes and impaired cardiac performance. One of the key pathological mechanisms is the disruption of glycogenolysis, which negatively impacts myocardial energy metabolism. Considering the critical importance of maintaining energy homeostasis in the heart, biologically active substances derived from cryopreserved xenogeneic heart fragments emerge as a promising therapeutic avenue.

Objective. To investigate the effects of an extract from cryopreserved piglet heart fragments on glycogenolysis activity in cardiomyocytes and its potential therapeutic impact in a model of adrenaline-induced myocardiodystrophy (AMD).

Methods. The study involved 84 outbred male rats (250–300 g) maintained under standard vivarium conditions. AMD was induced using a single subcutaneous injection of 0.18% adrenaline tartrate solution at a dose of 5 mg/kg. The experimental group received daily intraperitoneal injections of the extract at 50 µg of peptides per 100 g of body weight for 14 days. The control group was administered an equivalent volume of 0.9% sodium chloride solution. Amiodarone (10 mg/kg, intramuscularly) served as a reference drug. Heart tissue homogenates were analyzed post-decapitation. Glycogen content was measured using the glucose oxidase method, and glucose-6-phosphate (G-6-P) levels were determined spectrophotometrically using the hexokinase method.

Results. On day 2, rats treated with the extract showed a glycogen level of 3.1±0.14 mg/g (95% CI: 2.8–3.4), a 48.3% increase compared to controls ($p=0.007$). In the amiodarone group, glycogen reached 4.2±0.06 mg/g (95% CI: 4.1–4.3), a 99.3% increase over controls ($p<0.001$), yet lower than that in the extract group. By day 14, extract-treated rats

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exhibited a glycogen level of 8.0±0.30 mg/g (95% CI: 7.4–8.6), up 156.4% from day 2 (p=0.01) and 61.1% from day 7 (p=0.01).

On day 14, G-6-P levels in the control group were 0.79 [0.77–0.86] µmol/g, representing a 75.6% increase from day 2 (p=0.01) and 38.6% from day 7 (p=0.05). In the extract group, G-6-P levels reached 0.80 [0.79–0.81] µmol/g, a 56.9% rise from day 2 (p=0.01) and 25.0% from day 7 (p=0.01). The amiodarone group showed G-6-P levels of 0.82 [0.81–0.82] µmol/g, a 57.7% increase from day 2 (p=0.01) and 24.2% from day 7 (p=0.01).

Conclusions. The extract from cryopreserved piglet heart fragments demonstrated a significant corrective effect on carbohydrate metabolism disorders in the myocardium of rats with adrenaline-induced myocardiodystrophy. This includes normalization of glycogen and G-6-P levels, highlighting its potential as a therapeutic agent for myocardial ischemic and hypoxic conditions.

Keywords: extract of cryopreserved heart fragments, adrenaline-induced myocardiodystrophy, glycogenolysis, glucose-6-phosphate.

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КРІОЕКСТРАКТ СЕРЦЯ ЯК МОДУЛЯТОР ГЛІКОГЕНОЛІЗУ В УМОВАХ ЕКСПЕРИМЕНТАЛЬНОЇ МІОКАРДІОДИСТРОФІЇ

Актуальність. Міокардіодистрофія є серйозним порушенням обміну речовин у серцевому м'язі, що призводить до структурно-функціональних змін у кардіоміоцитах та порушення нормальної роботи серця. Одним із ключових аспектів патології є порушення процесів глікогенолізу, що негативно впливає на енергетичний обмін міокарда. Враховуючи важливість підтримки нормального енергетичного балансу в серці, одним із перспективних напрямів є використання біологічно активних речовин, отриманих з кріоконсервованих фрагментів ксеногенних сердець.

Мета роботи – вивчення впливу екстракту кріоконсервованих фрагментів серця поросят на активність глікогенолізу в кардіоміоцитах та його потенційний терапевтичний ефект на моделі адреналінової міокардіодистрофії (АМД).

Матеріали та методи. Експериментальні дослідження проведені на 84 нелінійних щурах-самцях масою 250–300 г, які утримувались у стандартних умовах віварію. АМД моделювали за методикою Маркової О.О. шляхом одноразового підшкірного (п/м) введення 0,18% розчину адреналіну тартрату в дозі 5 мг/кг маси тіла. Кріоконсервовані фрагменти серця поросят вводили внутрішньоочеревно з розрахунку 50 мкг пептидів на 100 г маси тварини щоденно протягом 14 днів. Тварини контрольної групи отримували еквіоб'ємну кількість 0,9% розчину натрію хлориду. В якості референс-препарату обрано аміодарон (10 мг/кг, в/м). Матеріалом дослідження виступали гомогенат тканин серця щурів після декапітації тварин. Матеріалом дослідження виступали гомогенат тканин серця щурів після декапітації тварин. Вміст глікогену визначали глюкозооксидазним методом. Вміст глюкозо-6-фосфату (Г-6-Ф) у тканинах серця визначали спектрофотометрично гексокіназним методом.

Результати та їх обговорення. У групі щурів, яким вводили

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ЕКСП, рівень глікогену був 3,1±0,14 мг/г (95% ДІ: 2,8–3,4), що на 48,3% більше, ніж у контрольних щурів (p=0,007). У групі з аміодароном рівень глікогену становив 4,2±0,06 мг/г (95% ДІ: 4,1–4,3), що на 99,3% більше порівняно з контролем (p<0,001), але був меншим, ніж в групі ЕКСП. На 14 день у групі щурів, які отримували ЕКСП, рівень глікогену був 8,0±0,30 мг/г (95% ДІ: 7,4–8,6), що на 156,4% більше порівняно з 2 днем (p=0,01) та на 61,1% більше порівняно з 7 днем (p=0,01).

На 14 день рівень Г-6-Ф у контрольних щурів був 0,79 [0,77; 0,86] мкмоль/г, що на 75,6% більше порівняно з 2 днем (p=0,01) та на 38,6% більше порівняно з 7 днем (p=0,05). У групі щурів, які отримували ЕКСП, рівень Г-6-Ф становив 0,80 [0,79; 0,81] мкмоль/г, що на 56,9% більше порівняно з 2 днем (p=0,01) та на 25,0% більше порівняно з 7 днем (p=0,01). У групі з аміодароном рівень Г-6-Ф був 0,82 [0,81; 0,82] мкмоль/г, що на 57,7% більше порівняно з 2 днем (p=0,01) та на 24,2% більше порівняно з 7 днем (p=0,01).

Висновки. Результати дослідження вказують на те, що ЕКСП має виражену коригувальну дію на порушення вуглеводного обміну в серці щурів з моделлю АМД. Цей ефект включає нормалізацію рівня глікогену і Г-6-Ф, що робить ЕКСП перспективним препаратом для лікування патологій, пов'язаних з ішемією та гіпоксією міокарда.

Ключові слова: екстракт кріоконсервованих фрагментів серця, адреналінова міокардіодистрофія, глікогеноліз, глюкозо-6-фосфат.

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INTRODUCTION

Myocardial dystrophy is accompanied by disturbances in myocardial energy metabolism, ultimately leading to a decline in the heart's ability to perform its pumping function. One of the key processes regulating energy metabolism in the heart is glycogenolysis – the breakdown of glycogen into glucose, which serves as an energy source for cells. In the context of myocardial dystrophy, glycogenolysis in the myocardium becomes a critical aspect for studying metabolic disturbances. Under normal physiological conditions, the heart primarily relies on aerobic metabolism for energy production; however, during ischemia or hypoxia, a shift toward anaerobic glycolysis occurs. This metabolic shift is accompanied by the activation of glycogenolysis to ensure energy supply to the myocardium [1].

During ischemia or hypoxia, the heart experiences elevated levels of catecholamines and cyclic AMP (cAMP), which stimulate the activity of phosphorylase A, the key enzyme of glycogenolysis. This facilitates glycogen mobilization to meet the myocardium's energy demands [2]. In the setting of myocardial dystrophy, this process may be impaired, further exacerbating the functional state of the heart.

Despite the activation of glycogenolysis, prolonged ischemia leads to a rapid depletion of glycogen stores. This results in decreased levels of ATP and creatine phosphate, thereby impairing myocardial function. Intracellular acidosis caused by lactate accumulation inhibits the activity of key glycolytic enzymes, such as phosphofructokinase. The resulting energy deficit disrupts membrane permeability, promoting the efflux of ions—particularly potassium—from the cell and the release of intracellular enzymes, potentially contributing to further myocardial damage [3].

Pharmacological interventions aimed at normalizing metabolic processes may have a beneficial effect on myocardial function [2]. Several studies suggest that the use of certain drugs can modulate myocardial metabolism. For instance, thiotriazoline exhibits antioxidant and membrane-stabilizing properties, which may contribute to the restoration of energy metabolism in the heart. At the same time, carvedilol, when used in combination with thiotriazoline, has been shown to reduce the activity of glycolytic enzymes, indicating a potential optimization of myocardial energy supply [4].

Amiodarone is known for its cardioprotective properties, which may influence metabolic processes in the heart, including glycogen mobilization. Although

the direct effect of amiodarone on glycogenolysis in myocardial dystrophy has not been thoroughly investigated, there is indirect evidence of its impact on cardiac metabolism. Amiodarone may affect both cardiac hemodynamics and cellular metabolism, which in turn could modulate glycogen mobilization in cardiomyocytes.

In the search for effective therapeutic strategies to correct metabolic disturbances in the heart, one promising approach involves the use of biologically active substances of natural origin, particularly extracts derived from cryopreserved mammalian organ fragments [5, 6]. Extraction using appropriate techniques allows for the preservation of the biological activity of the components, opening new avenues for the development of pharmacological agents with high bioactivity. Cardiac tissue cryoextracts contain a broad range of bioactive molecules capable of exerting regulatory effects on metabolic processes, including glycogenolysis.

The investigation of the efficacy of extracts derived from cryopreserved porcine heart fragments (ECPH) in the context of myocardial dystrophy, particularly their influence on glycogenolysis, represents a relevant and promising area of cardiological research, as it may lead to the development of novel therapeutic approaches for cardiovascular diseases.

The aim of the study was to examine the effect of the extract from cryopreserved porcine heart fragments on glycogenolysis activity in cardiomyocytes and to assess its potential therapeutic impact in an experimental model of adrenaline-induced myocardial dystrophy (AMD).

MATERIALS AND METHODS

Experimental studies were conducted on 84 outbred male rats weighing 250–300 g, which were housed under standard vivarium conditions.

Adrenaline-induced myocardial dystrophy (AMD) was modeled according to the method described by O.O. Markova (1998) by a single subcutaneous (s.c.) injection of 0.18% adrenaline tartrate solution (PJSC "Pharmaceutical Firm Darnitsa," Ukraine) at a dose of 5 mg/kg body weight [7].

Cryopreserved porcine heart fragments were obtained according to the protocol described in [5]. The concentration of peptides in the extracts was determined spectrophotometrically at a wavelength of 280 nm. The final peptide concentration in the extract was 0.1 mg/mL. The porcine heart extracts were administered intraperitoneally at a dose of 50 μg of peptides per 100 g of body weight once daily for 14 days.

Animals in the control group received an equivalent volume of 0.9% sodium chloride solution (NaCl, *Galichpharm, Ukraine*).

Amiodarone (10 mg/kg, intramuscularly; *Sanofi-Aventis, Ukraine*) was selected as the reference drug due to its well-documented profile of pleiotropic cardioprotective effects. Although amiodarone is classified in clinical practice as a class III antiarrhythmic agent according to the Vaughan Williams classification (1970) and is primarily used in patients with structural myocardial damage (e.g., left ventricular hypertrophy, myocardial infarction, or heart failure with reduced ejection fraction), a number of experimental studies have demonstrated its ability to modulate not only electrophysiological but also metabolic processes in cardiomyocytes [8]. Specifically, amiodarone exerts anti-ischemic effects, reduces oxidative stress, stabilizes cellular membranes, influences calcium homeostasis, and may contribute to the preservation of intracellular glycogen under hypercatecholaminemic conditions.

The choice of amiodarone as a reference compound in the model of adrenaline-induced cardiomyopathy is justified by its pathophysiological relevance: this model is characterized by excessive β-adrenergic stimulation, hypermetabolic stress, ischemia-reperfusion injury, and energy deficit – all of which represent conditions under which amiodarone is known to exert protective effects.

The material for analysis consisted of rat heart tissue homogenates obtained after decapitation. Glycogen content was determined using the glucose oxidase method [9]. The level of glucose-6-phosphate (G-6-P) in heart tissue was measured spectrophotometrically using the hexokinase method. In this reaction, glucose is converted by hexokinase in the presence of ATP into G-6-P, which is subsequently transformed into 6-phosphoglucono-D-lactone by G-6-P dehydrogenase. The amount of NADPH generated in this reaction is proportional to the glucose content in the sample and is determined by measuring light absorbance at a wavelength of λ = 340 nm. The G-6-P content was expressed in μmol/g [10].

All experimental procedures involving laboratory rats were carried out in accordance with the Law of Ukraine "On the Protection of Animals from Cruelty" No. 3477-IV dated February 21, 2006, the General Ethical Principles for Animal Experiments approved by the First National Congress on Bioethics of Ukraine (*Kyiv, 2001*), as well as other applicable national and international regulations.

The comprehensive research protocol was reviewed and approved by the Bioethics Committee at the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine (extract from Protocol No. 2, dated 3rd January 2022).

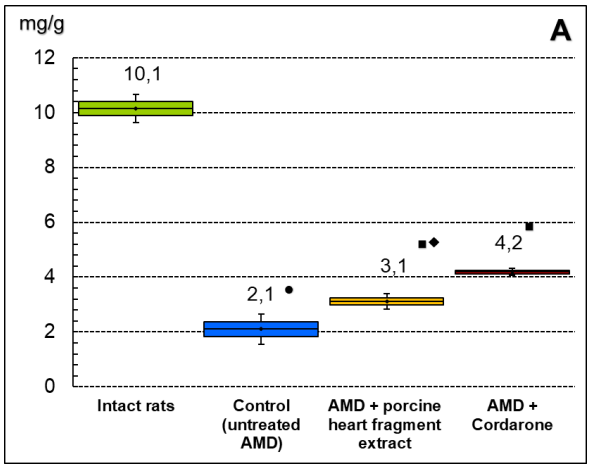
Statistical Analysis. Statistical processing of the obtained data was performed using the Microsoft Office Excel spreadsheet software. The distribution of variables within each sample group was assessed using

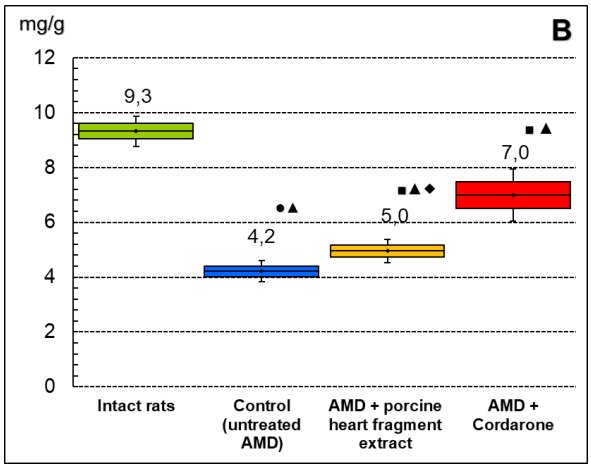
the Shapiro–Wilk W-test. Homogeneity of variances was evaluated using Levene's test.

In cases of normal distribution, the data are presented as "M ± m" (M ± SE), where M is the arithmetic mean and m (SE) is the standard error of the mean. The 95% confidence interval (CI) is provided as 5%–95%, where 95% CI refers to the 95% confidence interval.

RESULTS

Biochemical analyses demonstrated that on day 2 of the experiment, the glycogen level in the cardiac tissue of untreated control rats was reduced to 2.1 ± 0.28 mg/g (95% CI: 1.5–2.7), representing a 79.3% decrease compared to intact rats (10.1 ± 0.26 mg/g, $p < 0.001$) (Figure 1A).





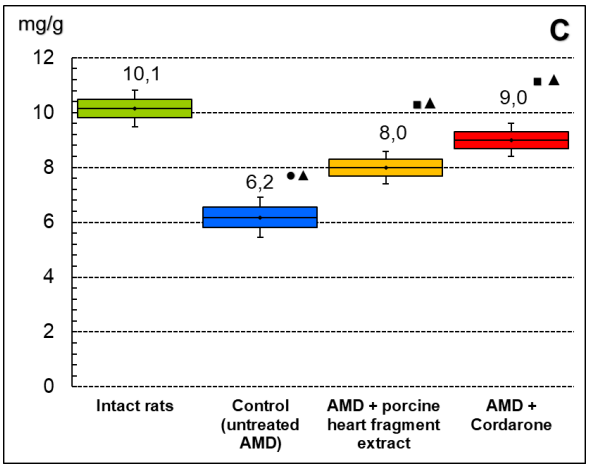


Fig. 1. Effect of cryopreserved porcine heart fragment extract on myocardial glycogen levels in rats with adrenaline-induced myocardial dystrophy, mg/g
(M±m, 95% CI, n=84; A – day 2, B – day 7, C – day 14)

Notes

1. The distribution of values within each sample group is normal.
2. Boxes represent the standard error of the mean; vertical whiskers indicate the 95% confidence interval.
3. The horizontal line within the box represents the arithmetic mean.
4. ● – $p < 0.05$ vs. intact animals.
5. ■ – $p < 0.05$ vs. untreated AMD animals (control group).
6. ▲ – $p < 0.05$ vs. day 2 of the experiment.
7. ◆ – $p < 0.05$ vs. AMD animals treated with the reference drug Cordarone

In the group of rats treated with the extract of cryopreserved porcine heart (ECPH), the glycogen level was 3.1 ± 0.14 mg/g (95% CI: 2.8–3.4), which was 48.3% higher than in the control group ($p = 0.007$).

In the amiodarone-treated group, the glycogen content reached 4.2 ± 0.06 mg/g (95% CI: 4.1–4.3), which was 99.3% higher compared to the control group ($p < 0.001$), but still lower than in the ECPH group.

On day 7 (Figure 1B), the glycogen level in the control group increased to 4.2 ± 0.19 mg/g (95% CI: 3.8–4.6), which was 100.7% higher compared to day 2 ($p = 0.009$). In the group treated with ECPH, the glycogen level reached 5.0 ± 0.22 mg/g (95% CI: 4.5–5.4), representing a 17.6% increase relative to the control group ($p = 0.03$). In the amiodarone group, the glycogen content was 7.0 ± 0.49 mg/g (95% CI: 6.0–7.9), which was 65.8% higher than in the control group ($p < 0.001$).

On day 14 (Figure 1C), the glycogen level in untreated rats (control group) reached 6.2 ± 0.37 mg/g (95% CI: 5.4–6.9), which was 193.9% higher compared to day 2 ($p = 0.01$) and 46.4% higher than on day 7 ($p = 0.01$). In the group treated with ECPH, the glycogen level was 8.0 ± 0.30 mg/g (95% CI: 7.4–8.6), representing a 156.4% increase versus day 2 ($p = 0.01$) and a 61.1% increase versus day 7 ($p = 0.01$). In the amiodarone-treated group, glycogen content was 9.0 ± 0.31 mg/g (95% CI: 8.4–9.6), which was 115.0% higher than on day 2 ($p = 0.01$) and 28.8% higher than on day 7 ($p = 0.01$).

Thus, administration of ECPH extract significantly increased glycogen levels in rat cardiac tissue at various

stages of the experiment. The effect of ECPH was evident throughout the study and was nearly comparable to that achieved with amiodarone.

The evaluation of the effect of cryopreserved porcine heart extract (ECPH) on glucose-6-phosphate (G-6-P) levels in rat cardiac tissue under the model of adrenaline-induced myocardial dystrophy (AMD) demonstrated that on day 2, the G-6-P level in the untreated control group was 0.45 [0.36; 0.47] µmol/g, which was 43.0% lower compared to intact rats (0.79 [0.74; 0.87] µmol/g, p = 0.001) (Figure 2A). In the group treated with ECPH, the G-6-P level was 0.51 [0.51; 0.54] µmol/g, which was 13.3% higher than in the control group (p = 0.004). In the amiodarone group, G-6-P levels reached 0.52 [0.52; 0.54] µmol/g, also 15.6% higher than in controls (p = 0.001).

On day 7 (Figure 2B), the G-6-P level in the control group increased to 0.57 [0.53; 0.80] µmol/g, representing a 26.7% increase compared to day 2 (p = 0.009). In the ECPH-treated group, the G-6-P level reached 0.64 [0.64; 0.65] µmol/g, 12.3% higher than in controls (p = 0.3). In the amiodarone group, G-6-P levels were 0.66 [0.63; 0.70] µmol/g, showing a 15.8% increase relative to the control group (p = 0.3).

On day 14 (Figure 2C), the G-6-P level in control rats was 0.79 [0.77; 0.86] µmol/g, which was 75.6% higher compared to day 2 (p = 0.01) and 38.6% higher than on day 7 (p = 0.05). In the group treated with ECPH, the G-6-P level was 0.80 [0.79; 0.81] µmol/g, representing a 56.9% increase compared to day 2 (p = 0.01) and a 25.0% increase compared to day 7 (p = 0.01). In the amiodarone group, the G-6-P level was 0.82 [0.81; 0.82] µmol/g, 57.7% higher compared to day 2 (p = 0.01) and 24.2% higher than on day 7 (p = 0.01).

Thus, administration of the extract of cryopreserved porcine heart fragments led to an increase in glucose-6-phosphate levels in rat cardiac tissue at all stages of the experiment, indicating a positive metabolic effect of this agent against the background of changes induced by adrenaline-induced myocardial dystrophy.

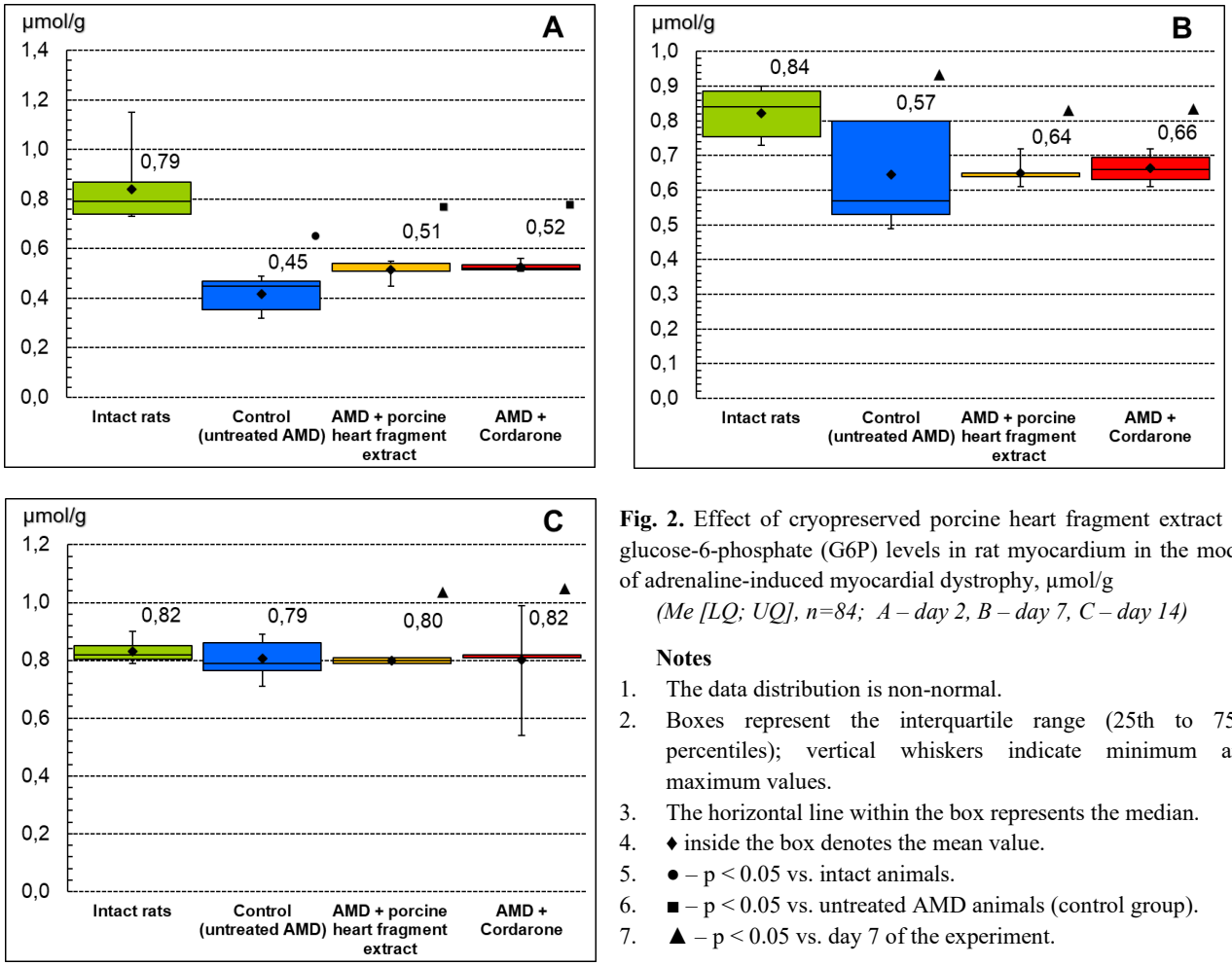


Fig. 2. Effect of cryopreserved porcine heart fragment extract on glucose-6-phosphate (G6P) levels in rat myocardium in the model of adrenaline-induced myocardial dystrophy, µmol/g

(Me [LQ; UQ], n=84; A – day 2, B – day 7, C – day 14)

Notes

1. The data distribution is non-normal.
2. Boxes represent the interquartile range (25th to 75th percentiles); vertical whiskers indicate minimum and maximum values.
3. The horizontal line within the box represents the median.
4. ♦ inside the box denotes the mean value.
5. ● – p < 0.05 vs. intact animals.
6. ■ – p < 0.05 vs. untreated AMD animals (control group).
7. ▲ – p < 0.05 vs. day 7 of the experiment.

DISCUSSION

It was established that adrenaline-induced myocardial dystrophy (AMD) is associated with marked disturbances in carbohydrate metabolism, manifested in particular by a significant decrease in myocardial glycogen levels at the early stages of the pathological process. This dynamic is characteristic of conditions accompanied by hypercatecholaminemia, impaired autonomic regulation, and the activation of stress-induced metabolic imbalance, which is typical of both toxic and ischemic myocardial injuries [11].

The rapid depletion of energy substrates, primarily glycogen, results from the activation of catabolic pathways triggered by excessive adrenaline, which—through elevated cyclic AMP—stimulates glycogenolysis and inhibits glycogenesis, thereby increasing glucose consumption and reducing its storage [12]. Moreover, glycogen deficiency in cardiomyocytes impairs the capacity for anaerobic energy production under ischemic conditions, further exacerbating metabolic disintegration and increasing the risk of structural cellular damage.

Against this background, administration of the ECPH extract led to a significant increase in myocardial glycogen levels as early as day 2 of the experiment, indicating early activation of mechanisms involved in the normalization of glucose metabolism. Such efficacy is consistent with the known properties of biologically active tissue-derived preparations, which contribute to the preservation of cell membrane integrity, maintenance of intracellular homeostasis, and stabilization of enzymatic activity – factors that are particularly important under conditions of oxidative stress and metabolic imbalance [13].

The results obtained show that glycogen levels in animals treated with ECPH remained consistently elevated throughout the study period, as evidenced by comparison with the control group, in which the values remained significantly lower. This dynamic supports the hypothesis that ECPH contributes to long-term metabolic adaptation and reduction of energy deficiency, aligning with current concepts regarding the actions of tissue extracts under conditions of metabolic stress [14].

Comparison with the reference drug, amiodarone, demonstrated that it induced slightly higher glycogen levels at all stages of the experiment. However, the difference between the two groups gradually decreased over time, suggesting a potential convergence in their metabolic efficacy. Similar effects of tissue extracts – capable of modulating both redox and energy metabolism, particularly through the restoration of mitochondrial function and activation of enzymatic systems – have been repeatedly reported in the literature [15]. These findings support the consideration of ECPH as a promising metabolic modulator for cardiotoxic injuries of various origins.

An additional indicator of carbohydrate metabolism status is the concentration of glucose-6-phosphate (G-6-P), the level of which reflects the activity of the hexokinase step of glycolysis. It was found that in the control group, a significant decrease in G-6-P levels was observed as early as day 2 of the experiment, indicating impaired glucose phosphorylation and the development of metabolic exhaustion characteristic of ischemic myocardial injury [16]. Conversely, administration of ECPH led to an increase in G-6-P levels, which can be interpreted as a restoration of hexokinase activity, enhancement of glycolytic flux, and improvement of the cell's energy supply.

The dynamics of G-6-P levels in the groups treated with ECPH or amiodarone were characterized by a steady increase throughout the experimental period. This indicates the prolonged action of both agents on glucose metabolism, supporting the functional state of cells under stress conditions [17].

The mechanisms underlying the effects of ECPH may be attributed to the presence of short regulatory peptides (RPs) that are part of the tissue matrix. These peptides exhibit high biological activity and may act as modulators of enzymatic systems, including those involved in glucose metabolism, as demonstrated in studies using extracts from other organs [18].

By day 14 of the experiment, both glycogen and G-6-P levels in rats treated with ECPH were significantly higher than those in the control group, indicating a sustained beneficial effect of the administered preparation. Similar findings have been reported in a model of doxorubicin-induced cardiomyopathy, where placental cryoextracts contributed to the preservation of cardiomyocyte functional activity by enhancing energy supply and reducing signs of cellular damage [19].

Complementing these findings, results of ultrasound investigations conducted on other experimental models have shown that cardiac tissue extracts exert beneficial effects on the morphofunctional characteristics of the myocardium. These include the preservation of ventricular wall thickness, improvement of ejection fraction, and reduction of remodeling manifestations. A correlation was established between the improvement of structural parameters and the normalization of biochemical markers of homeostasis – such as glycogen and G-6-P levels—which supports the efficacy of metabolic support strategies [20].

It is likely that the effects of ECPH are mediated through a combination of antioxidant and metabolic mechanisms, which is consistent with literature data showing that placental extracts can reduce the intensity

of lipid peroxidation, increase the activity of ATP-dependent systems, and promote the restoration of energy homeostasis under hypoxic or toxic conditions [21]. The relevance of this approach is further supported by current evidence on the cardioprotective effects of various anti-inflammatory biologically active agents, including disease-modifying drugs used in autoimmune disorders. Their effects are mediated through the reduction of oxidative stress, suppression of systemic inflammation, and improvement of energy metabolism in cardiomyocytes [22,23]. These results suggest that ECPH may represent a potentially effective agent for correcting energy deficits in toxic myocardial injury, with efficacy comparable to that of modern pharmacological agents, including amiodarone.

Thus, the presented data confirm the promise of using ECPH as a cell-free biological agent within combined therapeutic strategies for cardiotoxic conditions. Its mechanisms of action are presumably associated with the presence of short regulatory peptides (RPs), which, according to current evidence, are capable of specifically regulating the expression of genes involved in metabolic stability, antioxidant defense, and structural integrity of cells [24]. Such peptides are known to function as signaling molecules capable of activating transcription in specific regions of DNA, which is critically important for tissue repair and maintenance of cellular viability. In the context of cardiotoxic injury, this property enables not only a rapid metabolic response but also sustained preservation of myocardial functional activity, as evidenced by the biochemical marker dynamics observed in ECPH-treated rats.

CONCLUSIONS

1. Administration of ECPH to rats with adrenaline-induced myocardial dystrophy (AMD) resulted in a significant increase in myocardial glycogen levels as early as day 2, reaching 3.1 ± 0.14 mg/g – 48.3% higher compared to the control group ($p = 0.007$), indicating an early metabolic effect.

2. By day 14, glycogen content in ECPH-treated animals reached 8.0 ± 0.30 mg/g, representing a 156.4% increase relative to day 2 ($p = 0.01$) and a 61.1% increase compared to day 7 ($p = 0.01$), confirming the pronounced dynamic efficacy of ECPH in restoring myocardial energy reserves.

3. ECPH treatment led to a significant increase in glucose-6-phosphate (G-6-P) levels in cardiac tissue: on day 2 – by 13.3% compared to the control group ($p = 0.004$), and by day 14 – up to 0.80 [0.79; 0.81] μmol/g, exceeding the day 2 value by 56.9% ($p = 0.01$), indicating a recovery of glucose metabolism in response to ECPH.

PROSPECTS FOR FUTURE RESEARCH

The normalization of glycogen and glucose-6-phosphate (G-6-P) levels suggests that the extract of cryopreserved porcine heart fragments is a promising candidate for the treatment of myocardial pathologies associated with ischemia and hypoxia. The results of this experiment open new avenues for further research and potential clinical application of the extract in the management of cardiovascular diseases.

AUTHOR CONTRIBUTIONS

Chyzh M.O. – conceptualization and study design, execution of experimental research, statistical analysis of the obtained data, drafting of the main manuscript text, and formulation of conclusions.

Hladkykh F.V. – participation in experimental research, statistical analysis of the obtained data, drafting of the main manuscript text, and formulation of conclusions.

Liadova T.I. – contribution to the discussion of the results and critical revision of the manuscript.

Matviienko M.S. – statistical analysis of the obtained data, contribution to the discussion of the results, and manuscript editing.

Komorovsky R.R. – statistical analysis of the obtained data, contribution to the discussion of the results, and manuscript editing.

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CONFLICT OF INTEREST

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CONNECTION WITH OTHER SCIENTIFIC WORKS

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ARTIFICIAL INTELLIGENCE DISCLOSURE

The authors declare that no artificial intelligence (AI)-based technologies were used in the writing of the manuscript. AI tools were used exclusively for language editing and manuscript refinement.

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