Quantitative characteristics of structural changes in the myocardium of white rats during the modeling of adrenaline myocardiodystrophy and its pharmacological correction

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**ARTICLE INFO**
Received: 01 March 2023
Accepted: 03 April 2023

**UDC:** 616.127-007.17:577.175.522]-085.22-092.9

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**CONFLICT OF INTEREST**
The authors have no conflicts of interest to declare.

**FUNDING**
Not applicable.

**Introduction**
Pathology of the cardiovascular system is one of the leading causes of mortality and disability. In anesthesiology practice, it is the most common comorbid condition that leads to perioperative complications and fatal outcomes. The aim of the study was to substantiate the cardioprotective properties of succinic acid, sodium oxybutyrate, and quercetin based on the study of the myocardial morphological structure in the correction of experimental cardiac pathology. The experiments were conducted on white rats, in which adrenaline-induced myocardial dystrophy was modeled. The correction was performed using succinic acid, sodium oxybutyrate, and quercetin. Morphological studies were conducted at 2 and 24 hours after correction. At 2 hours after adrenaline administration, pronounced venous and arterial congestion was observed in the myocardium. The lumen of vessels appeared dilated, and their walls appeared thinner. In animals that received corrective agents after adrenaline, a positive effect of their application was noted. Vascular congestion was significantly reduced. The arterial walls had normal thickness, and the internal elastic membranes were moderately tortuous. No significant changes were observed in cardiomyocytes. At 24 hours from the start of pathology modeling and its pharmacological correction, the changes in the myocardium of the animals were much more pronounced compared to those with a 2-hour exposure, although in animals treated with corrective agents, the changes were less pronounced than in untreated animals. Rats exposed only to adrenaline showed significant trophic disturbances in cardiomyocytes, which developed against the background of coronary circulation disorders, manifested as wall thickening and narrowing of arterial lumens. The veins were congested, and blood extravasation was observed in the interstitium. Perivascular spaces expanded due to edema. Cardiomyocytes exhibited dystrophic changes, including areas with clarified cytoplasm, often with its homogenization and karyolysis. Histological data were morphometrically confirmed by changes in the Wogenworth index and nuclear-cytoplasmic ratios. The obtained results indicate the positive effect of the used corrective agents. Quercetin demonstrated slightly higher efficacy in this regard.

**Keywords:** myocardial dystrophy, correction, cardiomyocytes, arteries, veins.
However, the majority of modern scientific studies focusing on the impact of correction methods on the heart in its pathological state have a functional nature. At the same time, the number of studies with morphological investigations of their effectiveness, particularly in terms of quantitative characteristics that provide objective confirmation of structural changes occurring in the myocardium, remains insufficient [15].

The aim of the study is to substantiate the cardioprotective properties of succinic acid, sodium oxybutyrate, and quercetin based on the investigation of the morphological structure of the myocardium during the correction of experimental cardiac pathology.

Materials and methods

The experiments were conducted on 54 white laboratory non-pedigreed rats weighing 180-250 g.

The modeling of myocardial damage with the development of signs of cardiovascular insufficiency was carried out using the well-known technique by O.O. Markova [14]. The correction of the disorders was performed by administering succinic acid (at a dosage of 15 mg/kg), sodium oxybutyrate (at a dosage of 100 mg/kg), and quercetin (at a dosage of 1.33 ml/kg) through the enteral route. The drugs were administered 15 minutes after the start of the pathological process simulation. Morphological studies were conducted at 2 and 24 hours after the administration of the corrective agents, corresponding to the onset and peak of myocardial dystrophic lesions [5].

All experimental studies were conducted in accordance with the basic principles of the Resolution of the First National Congress on Bioethics "General Ethical Principles of Experiments on Animals", the Council of Europe Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, and the Helsinki Declaration.

Histological sections were stained with hematoxylin and eosin, as well as Van Gieson-Weigert stain. The quantitative assessment of vascular reactions was performed by calculating the Wogenwort index (WI), which represents the ratio of the arterial wall area to its lumen area [16]. The functional state of cardiomyocytes was evaluated based on the nuclear-cytoplasmic ratios (NCR) in the left ventricular myocardium [15].

The statistical analysis of the obtained data was performed using the Statsoft STATISTICA software package. The differences between the comparative values was determined using the Student's t-test.

Results

Two hours after the administration of a toxic dose of adrenaline, histological sections of the myocardium from experimental animals showed pronounced venous and arterial congestion. The lumen of the blood vessels appeared dilated, while their walls appeared thinner. The internal elastic membranes of the arteries were smoothed. Perivascular spaces were hardly discernible (Fig. 1). This indicated an increased blood flow to the myocardium.

As for the animals to which corrective agents were administered after adrenaline, in all cases, a positive effect of their application could be observed. The vascular congestion was significantly reduced. The arterial walls had normal thickness, and the internal elastic membranes were moderately tortuous. Small aggregates of erythrocytes were observed in the lumens.

At the same time, there were no significant morpho-functional changes observed in cardiomyocytes. Their cytoplasm exhibited normal staining, and the nuclei were well-defined.

During the morphometric studies, it was found that after 2 hours of isolated administration of adrenaline, the expansion of the arterial lumen was significantly confirmed by a 23.9 % decrease in the Wogenwort index (p<0.05) compared to the control group (Table 1). Similar reactions were observed in animals that received pharmacological correction after adrenaline administration. However, in these animals, the Wogenwort index showed only a tendency to decrease. Specifically, when quercetin was applied, the degree of decrease in the Vogenwort index was 7.5 %, for sodium oxybutyrate - 10.8 %, and for succinic acid - 11.4 % (p>0.05 in all cases). At the same time, in all cases of correction, the Wogenwort index remained significantly higher than the level observed in animals that received adrenaline alone: by 21.6 % with quercetin correction, by 17.2 % with sodium oxybutyrate correction, and by 16.4 % with succinic acid correction (p<0.05 in all cases).

Such vascular reactions with corresponding arterial and venous congestion had a certain influence on the morphofunctional state of cardiomyocytes. Despite practically unchanged nuclear area of cardiomyocytes, the area of surrounding cytoplasm significantly increased (Table 2), which could indicate enhanced cellular activity, particularly during the initiation of adrenaline-induced myocardial dystrophy modeling. Lumen of an intramural branch of the coronary artery - 1, erythrocytes in the lumen of a branch of the coronary vein - 2. Hematoxylin and eosin staining. x140.

Fig. 1. Histological section of the rat heart wall two hours after the initiation of adrenaline-induced myocardial dystrophy modeling. Lumen of an intramural branch of the coronary artery - 1, erythrocytes in the lumen of a branch of the coronary vein - 2. Hematoxylin and eosin staining. x140.
in the isolated administration of adrenaline, and could also be an early sign of their hydropic degeneration. The cytoplasmic area of cardiomyocytes, when corrected with succinic acid, sodium oxybutyrate, and especially quercetin, increased to a lesser extent. As a result, the nuclear-cytoplasmic ratio significantly decreased with isolated administration of adrenaline (by 11.3 % compared to the control, with p<0.05), while in combination with corrective agents, there was only a tendency towards reduction: by 6.3 % with succinic acid, 5.7 % with sodium oxybutyrate, and 4.6 % with quercetin (with p>0.05 in all cases). Meanwhile, compared to isolated administration of adrenaline, they significantly exceeded its level by 5.7 %, 6.4 %, and 7.8 %, respectively (with p<0.05 in all cases).

After 24 hours from the onset of modeling the studied pathology and its pharmacological correction, the changes in the myocardium of all experimental animals were significantly greater than in animals with a 2-hour exposure. Although in animals treated with corrective agents, these changes were less pronounced compared to animals without correction.

In rats exposed only to a toxic dose of adrenaline, pronounced disturbances in the trophism of cardiomyocytes were observed, which developed against the backdrop of severe disorders of coronary blood flow characterized by increased wall tone and narrowing of arterial lumen, confirmed by the tortuosity of their internal elastic membranes. Veins were congested, and blood extravasation was observed in the interstitium. Perivascular spaces expanded due to edema.

As a result of the disturbances in blood supply, cardiomyocytes exhibited changes of a dystrophic nature. Foci with clarified cytoplasm were frequently observed, and in some cases, even cytoplasmic homogenization and karyolysis occurred (Fig. 2).

These changes were supported by morphometric evidence. After 24 hours of isolated adrenaline administration, there was a significant increase in LV (left ventricle) on 28.2 % (p<0.05) compared to the control group, rather than a decrease, due to arterial constriction (see Table 1). Similar processes were observed in animals where medicinal correction was applied after adrenaline administration: LV increase with quercetin - by 8.8 % (p<0.05), sodium oxybutyrate - by 13.5 % (p<0.05), and succinic acid - by 15.9 % (p<0.05). However, in all correction cases, LV remained significantly lower than the level registered in the isolated administration of adrenaline, and could also be an early sign of their hydropic degeneration. The cytoplasmic area of cardiomyocytes, when corrected with succinic acid, sodium oxybutyrate, and especially quercetin, increased to a lesser extent. As a result, the nuclear-cytoplasmic ratio significantly decreased with isolated administration of adrenaline (by 11.3 % compared to the control, with p<0.05), while in combination with corrective agents, there was only a tendency towards reduction: by 6.3 % with succinic acid, 5.7 % with sodium oxybutyrate, and 4.6 % with quercetin (with p>0.05 in all cases). Meanwhile, compared to isolated administration of adrenaline, they significantly exceeded its level by 5.7 %, 6.4 %, and 7.8 %, respectively (with p<0.05 in all cases).

Table 1. Level of Wogenwort Index (WI) in intramural arteries of the left ventricle of white rats during adrenaline-induced myocardiodystrophy modeling and its correction (M±m).

<table>
<thead>
<tr>
<th>Values</th>
<th>Study group</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>2 hours WI</td>
<td>170.5±6.9</td>
</tr>
<tr>
<td>24 hours WI</td>
<td>170.6±6.9</td>
</tr>
</tbody>
</table>

Notes: * - p<0.05; ** - p<0.01 compared to the control; # - p<0.05 compared to adrenaline.

Table 2. Morphometric characteristics of left ventricular cardiomyocytes in the hearts of white rats during the modeling of adrenaline-induced myocardial dystrophy and its correction (M±m).

<table>
<thead>
<tr>
<th>Values</th>
<th>Study group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>2 hours LVCM</td>
<td>10.70±0.06</td>
</tr>
<tr>
<td>NCD</td>
<td>3.97±0.074</td>
</tr>
<tr>
<td>NCR</td>
<td>0.15±0.005</td>
</tr>
<tr>
<td>24 hours LVCM</td>
<td>10.70±0.06</td>
</tr>
<tr>
<td>NCD</td>
<td>3.97±0.072</td>
</tr>
<tr>
<td>NCR</td>
<td>0.15±0.005</td>
</tr>
</tbody>
</table>

Notes: * - p<0.05 compared to the control; ** - p<0.01 compared to the control; # - p<0.05 compared to adrenaline; LVCM - left ventricular cardiomyocyte diameter; NCD - nucleus cardiomyocyte diameter; NCR - nucleus-to-cytoplasm ratio.
animals receiving only adrenaline: by 21.6 % with quercetin correction, by 17.2 % with sodium oxybutyrate correction, and by 16.4 % with succinic acid correction (p<0.05 in all cases), which confirms the effectiveness of their corrective influence.

Against the backdrop of vascular reactions, corresponding changes were also observed in cardiomyocytes, which were quantitatively supported by morphometric research data. Despite almost unchanged nuclear area, the surrounding cytoplasm area significantly increased, particularly more intensely than during the 2-hour observation period (see Table 2), which may be a consequence of intracellular edema development. Moreover, the cytoplasm area increased more intensively specifically during isolated adrenaline administration. The cytoplasm area of cardiomyocytes increased to a lesser extent during succinic acid, sodium oxybutyrate, and especially quercetin correction, but at the same time, more intensively than during the 2-hour observation. As a result, the nuclear-cytoplasmatic ratio noticeably decreased in all cases: by 14.5 % (p<0.05) during isolated adrenaline administration, by 9.4 % (p<0.05) during succinic acid administration, by 8.2 % (p>0.05) during sodium oxybutyrate administration, and by 9.1 % (p<0.05) during quercetin administration compared to the control.

It should be noted that the difference in indicators between the corrected animals and animals with isolated adrenaline administration was no longer significant and exceeded its level by only 5.1-7.3 % (with p>0.05).

Discussion

As it is known, the development of most pathological processes is directly or indirectly associated with the disturbance of oxygen homeostasis in the body. In this regard, both general and local hypoxia are considered as additional factors of damage, initiating mechanisms of necrosis and oxidative stress [4].

Hypoxia itself induces a variety of pathological disorders. The main regulator of the body’s response to hypoxia is a specific protein found in cell nuclei called hypoxia-inducible factor (HIF-alpha). Under normal oxygen levels, the amount of HIF-alpha is low. However, when cellular oxygen levels sharply decrease, the level of HIF-alpha increases and can lead to cellular apoptosis. Suckinate, or in other words, succinic acid, is capable of inhibiting HIF-alpha and stabilizing its level [18].

Pharmaceutical preparations containing succinic acid have been used for a long time as antioxidants that normalize intracellular metabolism and promote the restoration of organ functions impaired by diseases or natural cell aging. The antihypoxic effects of succinic acid salts are enhanced by their antioxidant properties, their ability to modify phospholipids and facilitate their resynthesis, as well as reduce ion permeability of membranes and the efflux of K+ from mitochondria against the concentration gradient [2, 17].

Due to their broad pharmacodynamic spectrum and low toxicity, quercetin preparations have long attracted the attention of researchers and clinicians. The most important properties of these preparations are their pronounced antioxidant, immunomodulatory, and anti-inflammatory effects. Quercetin primarily acts as a scavenger of free radicals and has the ability to activate enzymes involved in the body’s own antioxidant defense. It exerts anti-inflammatory effects by blocking the lipooxygenase pathway of arachidonic acid metabolism, reducing the synthesis of leukotrienes, serotonin, and other inflammatory mediators. Quercetin enhances the activity of phagocytes, T- and B-lymphocytes, and the production of antibodies, thereby reducing the manifestations of secondary immunodeficiency [1, 10, 11].

Moreover, quercetin is one of the most common and readily available flavonoids, which accumulates in the form of glycosides in many medicinal plants [3, 8].

Sodium oxybutyrate belongs to the category of classical antihypoxants. It also exhibits elements of nootropic activity and exerts sedative, hypnotic, narcotic, and muscle-relaxing effects. Importantly, it enhances the body’s resistance, including that of the brain, heart, and retinal tissue, to hypoxia by activating oxidative processes [6].

Potentially, all antihypoxants, regardless of their chemical structure, can have secondary antioxidant effects due to their energy-stabilizing and antacid properties, which prevent excessive formation of free radicals and suppress endogenous ones [17].

Thus, the obtained results indicate a positive effect of the administered medications. In their selection, we focused on the fact that they share common antihypoxic properties. It is worth noting that quercetin showed slightly higher effectiveness compared to others due to its combination of cardioprotective and angioprotective properties [6, 9, 13].

The obtained results indicate the need for further study of cardioprotective agents with morphological justification of their effectiveness.

Conclusion

1. A cardiotoxic dose of adrenaline induces dystrophic changes in the cardiomyocytes of rats, which progressively increase from 2 to 24 hours of observation after administration of the drug.

2. Succinic acid, sodium oxybutyrate, and quercetin have a cardioprotective effect by exerting a positive regulatory influence on coronary blood flow and reducing myocardial hypoxia.

3. Quercetin is slightly more effective as a cardioprotective and angioprotective agent compared to succinic acid and sodium oxybutyrate.

References


Герасимюк К. О.

Патологія серцево-судинної системи є однією з основних причин смертності та інвалідизації. В анестезіологічній практиці патологію серцево-судинної системи відомо як основна причина смерті та інвалідизації.

Кількісна характеристика структурних змін в міокарді білих щурів при моделюванні адреналінової міокардіодистрофії

В патофізіологічних дослідженнях встановлено, що при генеруванні адреналінової міокардіодистрофії (АМД) у тварин відмічені значні структурні зміни в міокарді.

1. Більш вираженими були звичайні товщини середники, які піддалися лише впливу адреналіну.
2. Значно більшими були звичайні товщини внутрішніх еластичних мембран, які піддалися впливу адреналіну.
3. Внутрішні еластичні мембрани помірно звивисті.
4. У тварин, які піддалися впливу адреналіну, відмічені величезні зміни структурних властивостей кардіоміоцитів.

В суттєвому внеску до біологічних процесів внесли відкриття в сфері антиоксидантної терапії.

Відомо, що антиоксидантна терапія має високу ефективність при відновленні структурних та функціональних властивостей кардіоміоцитів у тварин.

Корекцію проводили після адrenаліну вводили коригуючі препарати, які моделювали адреналінову міокардіодистрофію.

Коректура проводили бутриновую кістку, оксібутрат матерію. В результаті проведених досліджень встановлено, що корекція проводить до значного підвищення структурних та функціональних властивостей кардіоміоцитів.

Важливою частиною антиоксидантної терапії є використання кверцетину.

Кверцетин є природним антиоксидантом і використовується для вивчення антиоксидантних властивостей в сфері міокардіодистрофії.


Вени були повнокровними, зустрічалися крововиливи в інтерстиції. Периваскулярні простори розширювалися за рахунок набряку. У кардіоміоцитах відмічалися зміни дистрофічного характеру: зустрічалися вогнища з просвітленою цитоплазмою, нерідко з її гомогенізацією й каріолізом. Гістологічні дані мали своє морфометричне підтвердження у вигляді відповідної динаміки індекса Вогенворта та ядерно-цитоплазматичних відношень. Отримані результати свідчать про позитивний ефект застосовуваних медикаментозних середників. Деяко більшу ефективність у цьому відношенні проявляє кверцетин.

Ключові слова: міокардіодистрофія, корекція, кардіоміоцити, артерії, вени.