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Analysis of mitochondrial-targeted antioxidant SkQ1 effectiveness on myocardial ischemia-reperfusion injury in a rat model: Focus on morphological and ultrastructural tissue study

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Mitochondrial-targeted antioxidant SkQ1 demonstrates a high efficiency in animal models and potentially can be used for minimizing postoperative complications in an on-pump open-heart surgery.

The aim of study was to the assessment of preservation degree and changes in the isolated rat heart characterized by prolonged cardioplegic ischemia, under the condition of donation of different SkQ1concentrations.

Material and methods. Isolated Langendorff-perfused rat hearts of Wistar line (*n*=15) were included in the presented study; the effectiveness of 12 ng/mL and 120 ng/mL of SkQ1 was analyzed. A biochemical analysis (superoxide dismutase 2 [SOD2], malondialdehyde [MDA], Troponin-I, heart-type fatty acid-binding protein [H-FABP]), a histological analysis of tissues (hematoxylin and eosin staining), scanning electron microscopy using backscattered electrons and immunofluorescence staining for cytochrome C and cytochrome P450 reductase were performed. The quantitative data were processed using GraphPad Prism 7 (Graph Pad Software, USA).

Results. The optimal myocardial preservation was discovered while using 12 ng/mL of SkQ1: the lowest concentrations of MDA (49.5 [41.1; 58.9] mmol/g), Troponin-I (22.3 [20.3; 23.9] pg/mL) and H-FABP (0.8 [0.6; 16.0] ng/mL) were associated with extensive areas of tissues with preserved transverse dark and light bands and a moderate interstitial edema. Moreover, non-deformed mitochondria were located mainly between the contractile fibers. Cytochrome C immunofluorescence was distributed locally, the luminescence intensity was 40% higher compared to the control group (p <0.0001). Increasing SkQ1 concentration to 120 ng/mL contributed to the aggravation of oxidative stress: MDA (63.8 [62.5; 83.0] mmol/g) and H-FABP (12.8 [4.1; 15.3] ng/mL) concentrations were closer to the control group values. Myocardial tissue in this group was characterized by a pronounced edema and a fragmentation of muscle fibers. There were signs of cardiomyocyte decay, myocytolysis and an intracellular edema. Cytochrome C was distributed evenly.

Conclusion. 12 ng/mL of SkQ1 demonstrates pronounced antioxidant effects in the ischemic myocardium leading to a higher degree preservation of the heart muscle compared to 120 ng/mL of SkQ1 that was associated with an aggravated oxidative stress and structural changes of the tissue.

Keywords: plastomitin; SkQ1; isolated rat heart; oxidative stress; ischemia-reperfusion injury; Langendorff perfusion; histology; scanning electron microscopy; immunofluorescence

Abbreviations: ROS – reactive oxygen species; ATP – adenosine triphosphate; SOD – superoxide dismutase; OS – oxidative stress; CVDs – coronary vascular diseases; AC – artificial circulation; SkQ1 – Skulachov's ions with plastoquinone; MDA – malondialdehyde; H-FABP – heart-type fatty acid-binding protein; ELISA – enzyme-linked immunoassay; SEM – scanning electron microscopy.

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Анализ эффективности действия митохондриально-направленного антиоксиданта SkQ1 на модели ишемии и реперфузии миокарда крыс. Акцент на морфологическом и ультраструктурном исследовании ткани

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Митохондриально-направленный антиоксидант SkQ1 демонстрирует высокую эффективность на разных экспериментальных моделях и может занять важное место в минимизации постоперационных осложнений при проведении операций на открытом сердце в условиях искусственного кровообращения или консервации трансплантата.

Цель. Оценить степень сохранности и изменений изолированного сердца крысы, которое подверглось длительной кардиоплегической ишемии, при условии донации разных концентраций SkQ1.

Материалы и методы. Исследование эффективности SkQ1 (12 нг/мл, 120 нг/мл и контрольная группа – без донации SkQ1) проведено на модели изолированного сердца крыс линии Wistar (*n*=15) по Лангендорфу. Провели биохимический анализ (супероксиддисмутазы 2 [СОД-2], малонового диальдегида [МДА] тропонин-I, сердечного белка, связывающего жирные кислоты [с-БСЖК]), гистологию ткани (окраска гематоксилин-эозином), сканирующую электронную микроскопию в обратно-рассеянных электронах и иммунофлуоресцентную окраску на цитохром С и редуктазу цитохрома Р-450. Количественные данные обрабатывали в программе GraphPad Prism 7 (GraphPad Software, США).

Результаты. Наибольшая сохранность ткани миокарда выявлена при поддержке SkQ1 в концентрации 12 нг/мл: наименьшие концентрации МДА (49,5 [41,1; 58,9] мкмоль/г), тропонин-I (22,3 [20,3; 23,9] пг/мл), с-БСЖК (0,8 [0,6; 16,0] нг/мл) логично сочетались с обширными зонами с сохранением поперечной исчерченности, умеренным интерстициальным отёком. Также выявлены недеформированые митохондрии, расположенные между сократительными волокнами, иммунофлуоресценция цитохрома С была распределена локально, интенсивность свечения на 40% выше в сравнении с контролем (p <0,0001). Увеличение концентрации SkQ1 до 120 нг/мл скорее способствовало усугублению окислительного стресса: концентрации МДА (63,8 [62,5; 83,0] мкмоль/г) и с-БСЖК (12,8 [4,1; 15,3] нг/мл) была ближе к контрольным значениям. Миокард данной группы охарактеризован резко выраженным отёком, фрагментацией мышечных волокон, некоторые группы кардиомиоцитов находились в состоянии глыбчатого распада, миоцитолиза и внутриклеточного отёка. Цитохром С был распределён равномерно в цитозоле кардиомиоцитов.

Заключение. SkQ1 в концентрации 12 нг/мл проявлял выраженные антиоксидантные свойства в отношении ишемизированного миокарда, что позволило получить более высокую степень сохранности сердечной мышцы в сравнении с применением SkQ1 в концентрация 120 нг/мл, которая усугубила окислительный стресс и структурные изменения ткани.

Ключевые слова: пластомитин; SkQ1; изолированное сердце крысы; окислительный стресс; ишемия-репефузия; перфузия по Лангендорфу; гистология; сканирующая электронная микроскопия; иммунофлуоресценция

Список сокращений: АФК – активные формы кислорода; АТФ – аденозинтрифосфат; СОД – супероксиддисмутаза; ОС – окислительный стресс; ССЗ – сердечно-сосудистые заболевания; ИК – искусственное кровообращение; SkQ1 – ионы Скулачёва с пластохиноном; МДА – малоновый диальдегид; с-БСЖК – сердечный белок, связывающий жирные кислоты; ИФА – иммуноферментный анализ; СЭМ – сканирующая электронная микроскопия.

INTRODUCTION

Antioxidants are molecules maintaining the cellular redox homeostasis via inhibiting reactions of free radicals with other biological molecules. Finally, antioxidants maintain the cell's function and its structural integrity [1]. Free radicals in living systems are represented mainly by reactive oxygen species (ROS; hydrogen peroxide, superoxide, singlet oxygen, hydroxyl radical, etc.) forming in mitochondria during the oxidative metabolism of adenosine triphosphate

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(ATP) generation [2, 3]. Antioxidants can be classified as endogenous (superoxide dismutase (SOD), catalase, glutathione peroxidase, α-tocopherol, glutathione, etc.) [4–6] and exogenous (carotenoids, flavonoids, lycopene, lutein, vitamins) [7–10].

Many pathological processes are accompanied by the increasing ROS production and oxidative stress (OS) development via a feedback mechanism [11, 12]. OS can be involved in the pathogenesis of various cardiovascular diseases [13]. OS burdens the myocardium during an open-heart surgery under the artificial circulation (AC), contributes to the postoperative recovery of patients [14] and plays an important role in the transplant suitability: preservation methods are currently being studied and improved [15, 16]. The violation in the "antioxidant–oxidant" balance requires additional antioxidant therapy; the deposition of antioxidants in mitochondria is a promising direction in the synthesis and study of mitochondria-targeted antioxidants.

In the late 1960s, Academician V.P. Skulachev synthesized the mitochondria-accumulated molecule of triphenylphosphonium. Based on this molecule, the whole class of compounds named "Skulachev ions" was finally developed [17].

The present study is devoted to one of the "strongest Skulachev ions" including a plastoquinone (SkQ1) as an antioxidant component [18], a decamethylene linker and a lipophilic cation that contribute to the successful penetration and incorporation of the antioxidant into the mitochondrial membrane [19–21]. Conducting an *ex vivo* experimental study on an isolated rat heart made it possible to model the clinical state of the myocardium that was subjected to IR, study changes at the tissue and cellular levels, and determine the most effective concentration of SkQ1.

THE AIM of study was to the assessment of preservation degree and changes in the isolated rat heart characterized by prolonged cardioplegic ischemia, under the condition of donation of different SkQ1concentrations.

MATHERIALS AND METHODS

Study design

The design flowchart is shown in Figure 1.

Study duration and conditions

The "Plastomitin" drug (SkQ1 concentrate – 1.7 mg/mL) was provided under a scientific cooperation agreement with Mitotech LLC (Moscow, Russia). The working solutions of SkQ1 were prepared by diluting the concentrate with a perfusion solution.

Experiments were carried out on healthy Wistar rats (♂, m=300±50 g, *n*=30) in accordance with the rules of the European Convention (Strasbourg, France, 1986). The animals were kept in standard vivarium conditions with free access to food and water. The daylight hours were 8 hours light and 16 hours without light at a humidity of 68%. The study was approved by the Local Ethics Committee of the Research Institute for Complex Issues of Cardiovascular Diseases (protocol No. 22 dated 10 Decemder 2015). The study was conducted from November to December 2022 at the scientific and technical base of the Research Institute for Complex Issues of Cardiovascular Diseases.

The perfusion of the isolated heart was performed according to Langendorff at a constant fluid column pressure of 80 cm H_2O. The Krebs-Henseleit solution containing 118.0 NaCl mmol/L, 25.0 mmol/L $NaHCO_3$, 11.0 mmol/L glucose, 4.8 mmol/L KCl, 1.2 mmol/L KH_2PO_4, 1.2 mmol/L $MgSO_4$ and 1.2 mmol/L $CaCl_2$, enriched with a gas mixture (95% O_2 and 5% CO_2, pH=7.4) with the temperature from 37 to 38°C, was used for the perfusion.

Perfusion protocol

The cardiac contraction stabilization (perfusion with Krebs-Henseleit solution) takes 20 min; connection of two flows of the perfusion solution with SkQ1 – 10 min; hypoperfusion (20 mL/h) with a cooled (t=4°C) cardioplegic solution (custodiol, Dr. F. KOHLER CHEMIE, GmbH, Germany) – 10 min; global cardioplegic ischemia – 240 min (t=8°C); a reperfusion – 30 min. Hearts included in the first experimental group were perfused with a double flow of the Krebs-Henseleit solution with 120 ng/mL SkQ1. Hearts included in the second experimental group were perfused similarly to the first experimental group with 12 ng/mL SkQ1.

Study groups

Three study groups were formed: the first experimental group "SkQ1 120 ng" (*n*=10), the second experimental group "SkQ1 12 ng" (*n*=10) perfused as it had been described above, and the control group (*n*=10) that was characterized by no SkQ1 supply to the perfusion solution.

Studied parameters

Biochemical parameters

Mitochondrial superoxide dismutase (SOD-2; EU2577, Wuhan Fine Biotech, China), heart-type fatty acid-binding protein (H-FABP; HK414, HycultBiotech,

the Netherlands), and cardiac troponin (troponin I; CSB-E08594r, CUSABIO BIOTECH Co., China) were measured by an enzyme-linked immunosorbent assay. Malondialdehyde (MDA) was determined using the OxiSelect™ TBARS Assay Kit MDA Quantitation (STA-330, Cell Biolabs, USA).

Histology

The explanted hearts were fixed in 10% buffered formalin (B06-001/M, BioVitrum, Russia) for 24 h, washed with water to remove the fixative solution and dehydrated in IsoPrep (06-002/M, BioVitrum, Russia) for 18 h. Then the samples were permeated with three portions of paraffin at 56°C for 60 min in each portion and embedded in HISTOMIX (247, BioVitrum, Russia). After this, 8 µm thick sections were prepared using the microtome (HM 325, Thermo Scientific, Waltham, MA, USA), placed in a thermostat and dried for 18 h at 37°C. Afterwards, the samples were dewaxed in three portions of o-xylene (103118, JSC LENREACTIV, Russia) for 1–2 min and dehydrated in three portions of 96% alcohol for 1–2 min. The sections were stained with Harris hematoxylin (05-004, BioVitrum, Russia,) for 15–20 min followed by washing up to 10 min; several drops of eosin (05-011/L, BioVitrum, Novosibirsk, Russia) were applied to the section for 30 sec. Then the samples were placed in 96% alcohol. Finally, the sections were cleared in o-xylene for several minutes followed by its subsequent removal. At the last stage, the sections were embedded in the mounting medium Vitrogel (12-005, BioVitrum, Russia) and examined by light microscopy using an AXIO Imager A1 microscope (Carl Zeiss, Oberkochen, Germany) at ×50, ×200 and ×400 magnification.

Scanning electronic microscopy

The samples were fixed in 4% buffered formalin for 24 h, postfixed with 1% osmium tetroxide in 0.1 M phosphate buffer (10010001, Gibco, USA) and stained with 2% osmium tetroxide (7563, Sigma-Aldrich, St. Louis, MO, USA) in bidistilled water for 48 h. Next, the samples were dehydrated in a series of increasing concentrations of ethanol (50, 60, 70, 80 and 95%), stained with 2% uranyl acetate (22400-2, Electron Microscopy Sciences, USA) in 95% ethanol, dehydrated with 99.7% isopropanol (06-002/M, BioVitrum, Novosibirsk, Russia) for 5 h and acetone (OKP241811ONO, Reachim, Staraya Kupavna, Russia) for 1 h, permeated with a mixture of acetone and Epon epoxy resin (14910, Electron Microscopy Sciences, USA) in a 1:1 ratio (6 h), transferred to a fresh portion of epoxy resin for 24 h and performed its polymerization in FixiForm containers (40300085, Struers, Denmark) at 60°C. Next, the samples in epoxy blocks were ground and polished using a TegraPol-11 unit (60799, Struers, Ballerup, Denmark). Contrasting with lead citrate was carried out in accordance with Reynolds for 7 min by applying the solution to the surface of the ground sample followed by washing it with bidistilled water. Then, carbon was sprayed onto the polished surface of the epoxy blocks (coating thickness was 10–15 nm) using a vacuum sputtering post (EM ACE200, Leica, Germany). The structure of the samples was visualized using the scanning electron microscopy in backscattered electrons on the electron microscope (S-3400N, Hitachi, Japan) in the BSECOMP mode at an accelerating voltage of 10 kV.

Confocal microscopy

Serial cryosections of 8 µm thickness were prepared from frozen rat myocardial samples (8 samples per heart) at 100 µm intervals using a cryotome (Microm HM 525, Thermo Scientific, USA). The preparations were fixed in a 4% paraformaldehyde solution for 10 min. For the permeabilization, the sections were treated with a 0.1% Triton-X100 solution (X100, Sigma-Aldrich, USA) for 15 min. Next, a mixture of primary antibodies (mouse antibodies to cytochrome C (ab13575, Abcam, UK) and rabbit antibodies to cytochrome P-450 reductase (ab180597, Abcam, UK) were applied to the sections and incubated for 18 h at 4°C. Then, the sections were washed and incubated with a mixture of secondary antibodies (goat antibodies to rabbit IgG conjugated with Alexa Fluor 488-conjugated (A11034, Thermo Fisher Scientific, USA) and goat antibodies to mouse IgG conjugated with Alexa Fluor 555-conjugated (A31570, Thermo Fisher Scientific, USA) for 1 h at room temperature. At all staining stages, phosphate-buffered saline supplied with 0.1% Tween 20 (P9416, Sigma-Aldrich, USA) was used. To remove autofluorescence, the sections were treated with Autofluorescence Eliminator Reagent (2160, Merck KGaA, Germany) according to the manufacturer's protocol. Cell nuclei were counterstained with DAPI (10 µg/mL, D9542, Sigma-Aldrich, USA) for 30 min. The prepared samples were cover-slipped using ProLong Gold Antifade Mountant (P36930, Thermo Fisher Scientific, USA). After drying, the samples were analyzed using the confocal microscope LSM700 (Carl Zeiss, Germany).

For each sample, 10 randomly selected fields of view were analyzed using ImageJ software (Wayne Rasband (NIH), USA) and the measured the average fluorescent signal intensity.

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Statistical analysis

The obtained data were analyzed using GraphPad Prism 7 software (GraphPad Software, USA). The data distribution was assessed using the Kolmogorov-Smirnov and Shapiro–Wilk tests. Quantitative data are presented as median (Me) and quartiles [25%; 75%]. Intergroup differences were assessed using the Kruskal–Wallis criterion with Dann's correction. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Biochemical analysis

SOD is one of the most important metal-containing enzymes involved in the cellular antioxidant system and an integral criterion for the evaluation of the OS level. Its mitochondrial isoform (SOD-2) can characterize the local antioxidant system of cells and a mitochondrial dysfunction [22–24]. In present experiment, after modeling of 240-minute cold cardioplegia, the SOD-2 concentration had no significant differences between the experimental and control groups (Table 1).

MDA is a product of oxidation of ω-3 and ω-6 fatty acids, a reactive aldehyde that covalently binds to lipids, proteins and DNA, thereby disrupting their function, it can be suggested as another OS marker [25–27]. The maximum amount of MDA per 1 g of myocardial tissue was found in the control group without any SkQ1 support and reached 70.9 [58.7; 87.8] μmol/g. The minimum MDA concentration was observed in the "SkQ1 12 ng" group 49.4 [41.1; 58.9] μmol/g, which was statistically significantly lower than the control values (p=0.02). H-FABP, located in the cytoplasm of the striated muscle cells, is quickly released in response to the damage, so it can be used as a sensitive marker of an ischemic myocardial injury [28, 29]. The median reflecting the level of released H-FABP into the myocardial outflow in the end of the reperfusion was lower in the "SkQ 12 ng group" (0.8 ng/mL) in comparison with other studied groups (12.8 ng/mL in the "SkQ 120 ng" group and 9.0 ng/mL in the control group), but this decreasing was not statistically significant. Cardiac troponin is a clinical biomarker for the diagnosis of the myocardial infarction [30]. The concentration of troponin-I was statistically significantly lower in the SkQ1 12 ng and SkQ1 120 ng groups compared to the control (p=0.03; Table 1).

So, the additional antioxidant support from 12 ng/mL SkQ1 leads to the OS inhibition and the decreasing release of myocardial injury markers under 240-minute cold cardioplegic ischemia followed by a reperfusion.

Histological analysis

The histological analysis data are correlated with the results of the biochemical analysis. The most significant structural changes in the myocardium (cardiomyocytes, vessels and stroma) were found in the control and "SkQ1 120 ng" groups. In the control group, a diffuse edema in the interstitium was noted (Fig. 2A). The vessels were paretically dilated, empty with swollen endothelium. The muscle fibers in the intramural zones were thinned, discomplexed, wavy deformed and unevenly stained. Extensive areas of the muscle fiber fragmentation in the subepicardial and subendocardial zones could be visualized. The cytoplasm of cardiomyocytes was homogeneous oxyphilic without a transverse striation (Fig. 2B).

Despite the established biochemical parameters, supplying of 120 ng/mL SkQ1 into the perfusion solution leads to significant structural changes. Myocardium is characterized by a pronounced edema (Fig. 2C) mainly in the perivascular spaces and intermuscular layers. Paretic dilation of vessels is accompanied with an endothelial detachment in some of them. The fragmentation and discomplexation of cardiomyocytes are more pronounced in the subepicardial and subendocardial zones. Fragmentation and depletion of the muscle fibers are combined with their waviness and polychromasia. The integrity of the sarcolemma is significantly impaired. Groups of cardiomyocytes characterized by a lumpy disintegration and myocytolysis, as well as an intracellular edema, have been discovered. The cell nuclei are deformed, unevenly enlarged and edematous, and characterized by a blurry outline and a diffuse enlightenment of chromatin. The focal lipofuscinosis has been noted (Fig. 2D).

The myocardium of the "SkQ1 12 ng" group was characterized by minimal structural changes. The extensive areas of compact cardiomyocytes arrangement alternated with the fiber fragmentation areas. Moderate edema, mainly in the perivascular zones, was noted in the interstitium. The nuclei of cardiomyocytes were clearly visible; they were located closer to the center of the cytoplasm, in which a transverse striation was preserved. However, the groups of the cells with swelling cytoplasm and poorly visible nuclei with unclear contours were visualized (Fig. 2E and 2F).

Thus, discirculatory, dystrophic and destructive changes in the myocardium were revealed in all the studied groups. However, these changes were less pronounced in the groups with an additional antioxidant support of the isolated hearts during the anoxia period by 12 ng/mL SkQ1 and had a mosaic character,

combined with extensive zones of relatively intact myocardium.

Scanning electron microscopy in backscattered electrons

High-magnification images of the samples obtained using scanning electron microscopy made it possible to analyze the intracellular changes in details. Without antioxidant support from SkQ1, the presence of zones with "erased" transverse striations was noted. In these zones, mitochondria "stuck" together and formed electron-dense structures that did not have a clear division into individual organelles in their structure. The nuclei were compressed, usually round in shape without a clear boundary, tightly adjacent to the electron-dense contents of the cytoplasm, the chromatin was located in a disordered manner (Fig. 3A and 3B). At high magnifications, a disruption of the structure of contractile fibers was observed, which did not have a clear structure, probably due to lysis (Fig. 3C). The space between the cells was expanded and filled with fibrous contents.

In the "SkQ1 120 ng" group, enlarged spaces between cardiomyocytes containing fragments of capillaries and cells were found (Fig. 3D). Cytoplasm of cardiomyocytes in the areas of the mitochondria localization was characterized by the presence of vacuole-like structures with transparent contents (Fig. 3E). Mitochondria had various shapes and a high electron density, some of them had surface outgrowths. These organelles often formed dense clusters and were adjacent to free spaces. In some areas, mitochondria had a tight contact with contractile fibers (Fig. 3F). In this group, the nuclei of cardiomyocytes were light, elongated, the nuclear membrane was uneven, the nucleoli and euchromatin were practically absent (Fig. 3E).

In the "SkQ1 12 ng" group, the transverse striation of the myocardial samples was discovered. The contractile fibers and cardiomyocytes were highly preserved (Fig. 3G–I). The mitochondria with clear boundaries were mostly located in the layers between the contractile fibers (Fig. 3 H, I). The nuclei of cardiomyocytes were elongated, the surface was smooth. Euchromatin was located parietal; the nucleoli were registered in the center of the nuclei. Free spaces were usually located between the outer surface of the nucleus and the contractile fibers.

Thus, a high structural and intracellular preservation of the myocardium treated by 12 ng/mL SkQ, was confirmed and detailed.

Confocal microscopy

Cytochrome C is a globular protein covalently linked to the heme group performing many important functions. It acts as an electron carrier from complex III to complex IV in the mitochondrial electron transport chain [31]. Cytochrome C, released from the mitochondria, leads to the apoptosis activation [32]. The distribution and intensity of cytochrome C fluorescence in the studied tissues differed depending on the SkQ1 concentration. The most increased immunofluorescence was noted in both groups supplied with SkQ1, the control group was characterized by the extremely low immunofluorescence (Figure 4A–C). Quantitative analysis confirmed the visual observations: cytochrome C fluorescence in the myocardial tissue from the "SkQ1 12 ng" group was statistically significantly higher compared to the control (p <0.0001, Fig. 3D). At the same time, no statistically significant differences between the experimental groups were found. It should be noted that 12 ng/mL SkQ1 supplying is associated with the most local increase in the luminescence of cytochrome C in the areas of structural preservation of the tissue. 120 ng/mL SkQ1 treatments led to the relatively uniform immunofluorescence of cytochrome C that may indicate the release of this enzyme into the cytosol.

Cytochrome P-450 reductase (POR) is a part of the cytochrome P-450 system localized in the membrane of the smooth endoplasmic reticulum. This enzyme is necessary for the transfer of electrons from NADP to cytochrome P-450 in microsomes, as well as to heme oxygenase and cytochrome B5 [33]. POR was poorly visualized in the tissue, and there was no statistical differences found between the studied groups (Fig. 3A–D).

Here, the concentration-depended effectiveness of the mitochondria-targeted antioxidant SkQ1 on the isolated rat heart model according to Langendorff has been demonstrated. The chosen model can help to directly access the effect of the studied antioxidant, its cardiotoxicity, excluding the influence of hormones, the autonomic nervous system and other organs [34]. The stages of cardiac perfusion had been constructed to model the clinical conditions during heart surgeries under artificial circulation or conditions close to the transplantation. The similar studies had already been previously conducted [35].

It has been found out that the 12 ng/mL SkQ1 is associated with the greatest preservation of the myocardium at the tissue and cellular levels. A histological examination and scanning electron microscopy revealed extensive zones with undeformed muscle fibers, intact sarcolemma and organelles of cardiomyocytes. These data were associated with the decreased levels of OS

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and myocardial injury markers. This concentration is probably triggering the binding of SkQ1 to cardiolipin followed by the decreasing peroxidase activity of cytochrome C, which accordingly led to the decreasing of OS in mitochondria, their structural preservation, which ultimately did not allow cytochrome C to enter the cytosol [36]. L.E. Bakeeva et al. have performed an *ex vivo* and *in vivo* study of the dose-dependent SkQ1 effect on Wistar rats and concluded that low SkQ1 concentrations can have a pronounced antioxidant effect in case of OS [37]. In 2021, M. Hamed et al. showed that 50 nmol/L MitoQ (SkQ analogue developed in New Zealand included ubiquinone in its structure) was associated with the pronounced antioxidant effect followed by an increase in the total blood flow and diuresis in the kidneys of pigs and humans [38].

In the present experiments, a 10-fold increase in the SkQ1 concentration (120 ng/mL) triggered OS in modeling an ischemic and reperfusion injury in the isolated heart. The study showed the extensive areas with deformed and disconnected muscle fibers, edema, lumpy decay (signs of acute tissue necrosis) with relatively high-level OS markers and a myocardial injury and, at the same time, continuous intracellular immunofluorescence of cytochrome C signaling a violation of the structure and function of mitochondria and possible apoptosis triggering. It can be concluded that perfusion of an isolated heart for 10 min with 120 ng/mL SkQ1 before the global ischemia has a cardiotoxic effect. The concentration of mitochondria-targeted antioxidant plays an important role in the correction of OS.

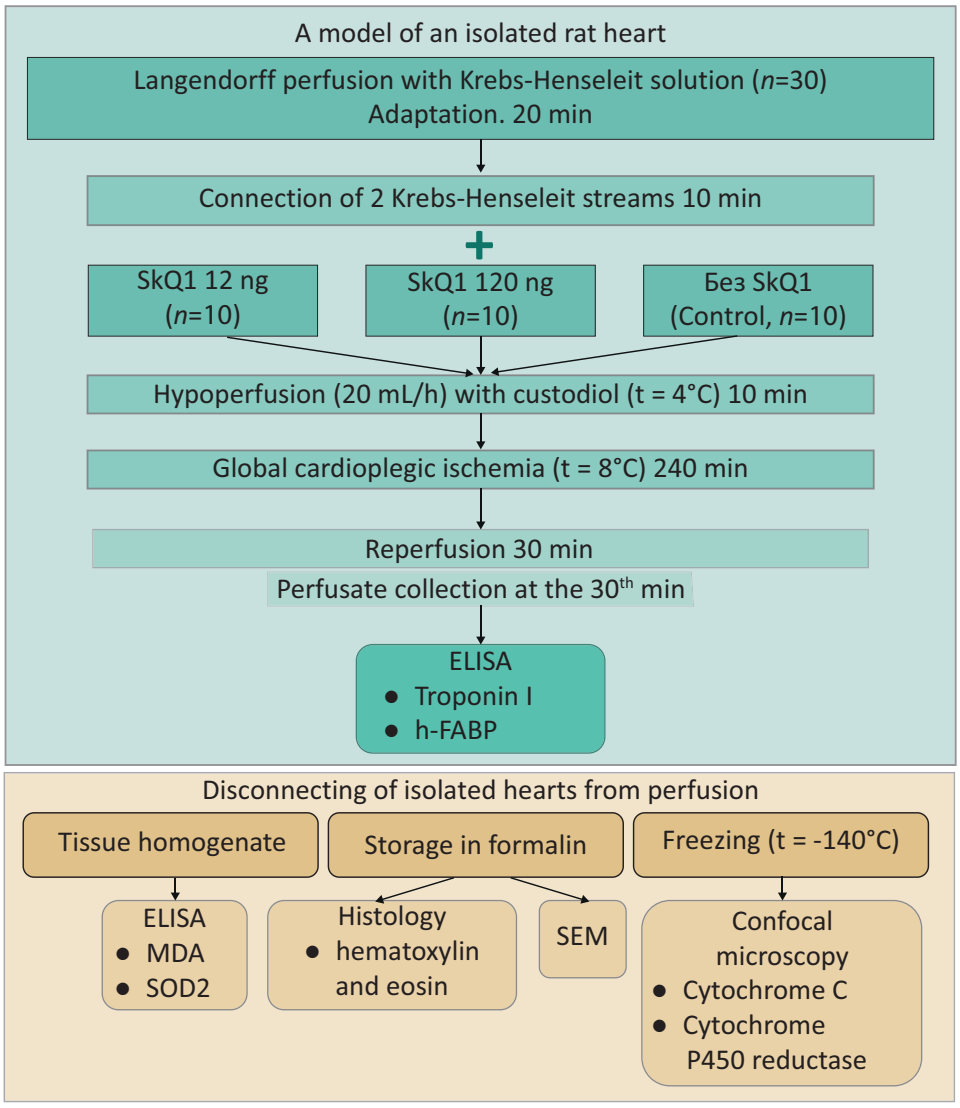

Figure 1 – Diagram of study design

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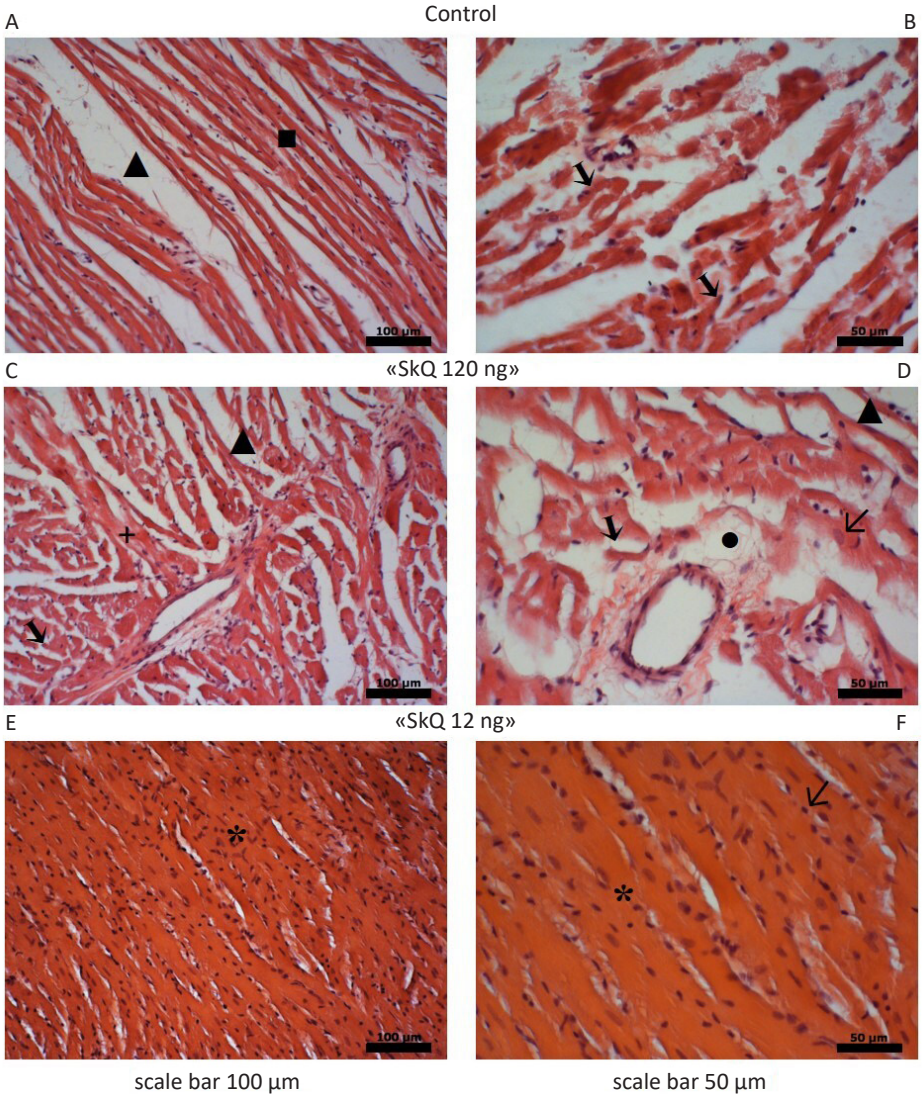

Figure 2 – Histological staining of myocardial sections with hematoxylin and eosin

Notes: A – diffuse interstitial edema (▲), stretching and thinning of muscle fibers (■). Magnification ×20; B ("SkQ1 120 ng") – fragmentation of muscle fibers, discomplexation of cardiomyocytes (↓), oxyphilia of the cardiomyocytes cytoplasm. Magnification ×40; C – disunion, fragmentation (↓) and polychromasia of muscle fibers, interstitial edema (▲), myocytolysis and intracellular edema of cardiomyocytes (+), magnification×20; D ("SkQ1 12 ng") – fragmentation of muscle fibers (↓), perivascular (●) and interstitial edema (▲). Swelling of cardiomyocyte nuclei (↓), magnification ×20; E – Compact arrangement of muscle fibers (*), magnification ×20; E – compact arrangement of muscle fibers (*), magnification ×20. F – compact arrangement of muscle fibers (*), well-distinguished nuclei of cardiomyocytes (↓), preservation of sarcolemma, magnification ×40.

Table 1 – Biochemical parameters of oxidative stress and myocardial injury

Groups	Study parameters, Me [25%; 75%]			
	SOD-2, ng/mL	MDA, µmol/g	Troponin-I, pg/mL	H-FABP, ng/mL
Control	13.0 [8.3; 18.3]	70.9 [58.7; 87.8]	47.7 [29.3; 54.2]	9.0 [2.1; 17.6]
SkQ1 120 ng	14.4 [11.6; 20.4]	63.8 [62.5; 83.0]	24.2 [23.5; 25.9]*	12.8 [4.1; 15.3]
SkQ1 12 ng	16.0 [8.4; 17.9]	49.5 [41.1; 58.9]*	22.3 [20.3; 23.9]*	0.8 [0.6; 6.0]

Note: * p <0,05 compared to the control group. SOD – superoxide dismutase; MDA – malondialdehyde; H-FABP – cardiac fatty acid binding protein.

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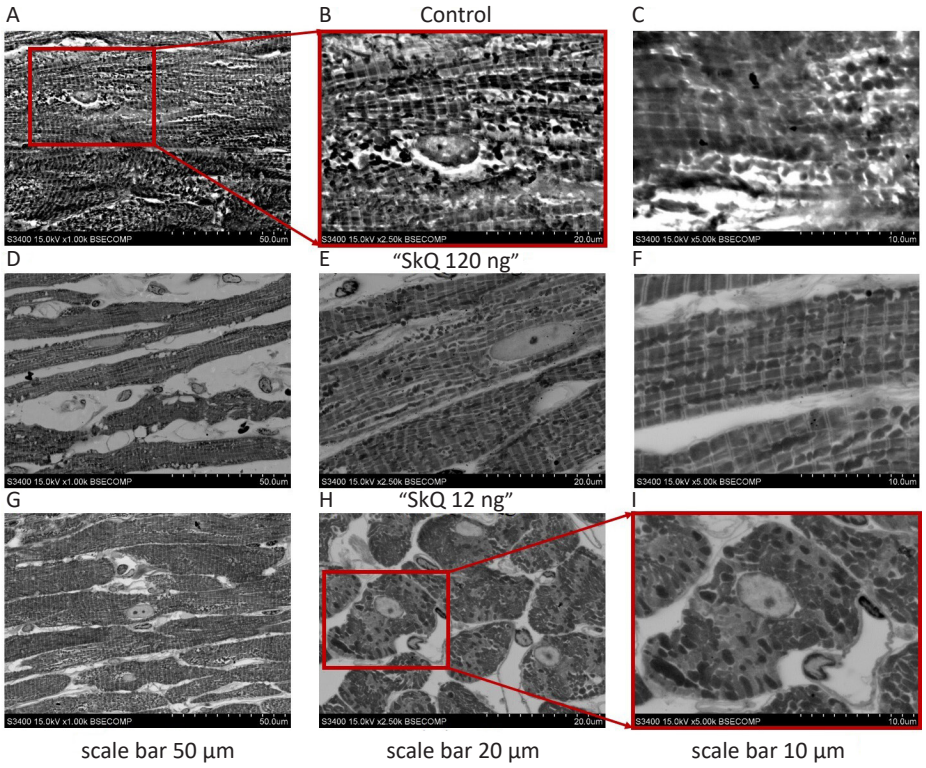

Figure 3 – Scanning electron microscopy of the myocardium

Notes: A – Wave-like deformation, zones of erased striation, magnification ×1000; B – Compressed nucleus, magnification ×2500; C – ("SkQ1 120 ng") – erased striation, disruption of the structure of contractile fibers, magnification×5000; D – dissociation of cardiomyocytes, magnification ×1000; E – Vacuole-like spaces around mitochondria, magnification ×2500; F – (SkQ1 12 ng) – clusters of mitochondria, magnification×5000; G – preservation of cardiomyocyte striation, magnification ×1000; H, I – layers of mitochondria around muscle fibers, elongated nuclei, magnification ×2500 and ×5000.

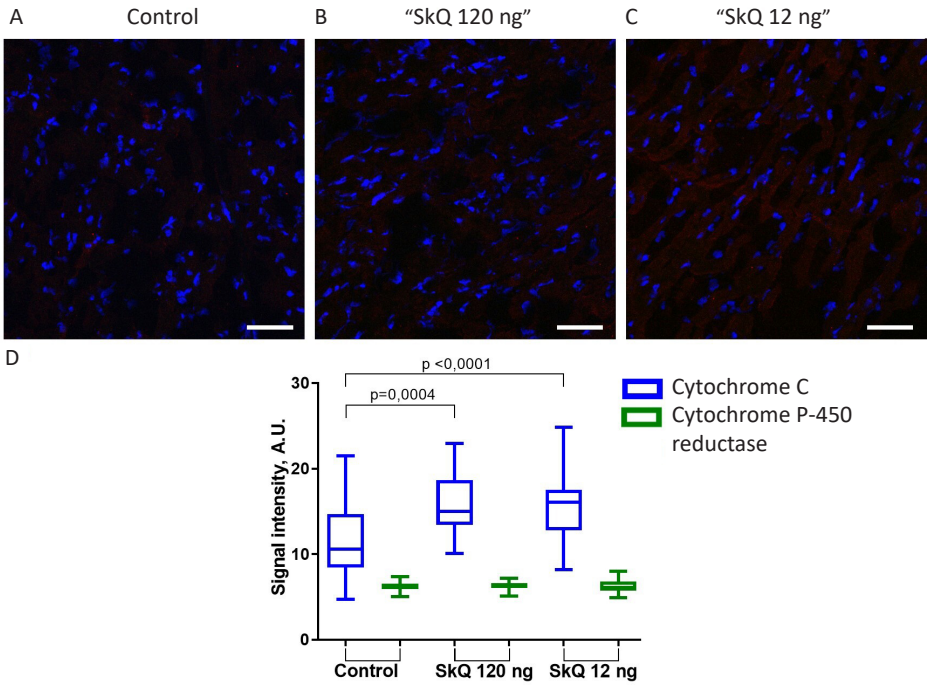

Figure 4 – Immunofluorescent staining of myocardial tissue sections

Notes: A – Control, weak fluorescence of immunofluorescent labels; B – SkQ1 120 ng, differentiated fluorescence of cytochrome C; C – SkQ1 12 ng, well-differentiated and zonal fluorescence of cytochrome C; D – quantitative analysis of cytochrome C and cytochrome P-450 reductase. The data are presented as a median with interquartile range and maximum and minimum values.Scale bar is 50 µm. Cytochrome C is red, cytochrome P-450 reductase is green, cell nucleus is blue.

Study limitations

The present study is limited by a small sample size (10 hearts in each study group). It should be noted that despite all the advantages and simplicity of the isolated heart model, the cut-off of a systemic influence could be also a limitation on the regulation of the cardiac muscle activity.

CONCLUSION

Mitochondria-targeted antioxidant SkQ1 (12 ng/mL) is associated with a pronounced antioxidant and cardioprotective effect on the model of ischemia and reperfusion on an isolated rat heart reflected in a high degree of preservation of the contractile apparatus of the myocardium and organelles. At the same time, a concentration of 120 ng/mL aggravated OS and led to destructive tissue damage. This antioxidant is extremely promising in the field of creating new cardioprotective drugs for a cardiac surgery and transplantation and requires further research.

At a concentration of 12 ng/mL, SkQ1 showed pronounced antioxidant properties against ischaemic myocardium, which resulted in a higher degree of cardiac muscle preservation compared with the use of SkQ1 at a concentration of 120 ng/mL, which exacerbated an oxidative stress and structural tissue changes.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS CONTRIBUTION

Evgeniya A. Senokosova, Evgeniy V. Grigoriev – study conception and design; Evgeniya A. Senokosova, Elena A. Velikanova, Rinat A. Mukhamadiyarov, Olga D. Sidorova, Evgeniya O. Krivkina, Larisa V. Antonova – research conducting, results processing and analyzing; Evgeniya A. Senokosova, Elena A. Velikanova – statistical analysis; Evgeniya A. Senokosova, Elena A. Velikanova, Rinat A. Mukhamadiyarov – manuscript preparation; Larisa V. Antonova, Evgeniy V. Grigoriev – manuscript revision. All authors confirm that their authorship complies with the international ICMJE criteria (all authors made a significant contribution to the concept development, research, and preparation of the article, read and approved the final version before publication).

REFERENCES

1. Soeur J, Eilstein J, Lereaux G, Jones C. Marrot L. Skin resistance to oxidative stress induced by resveratrol: from Nrf2 activation to GSH biosynthesis. Free Radic. Biol. Med. 2015;78:213–23. DOI: 10.1016/j.freeradbiomed
2. Cadenas E, Davies KJ. Mitochondrial free radical generation, oxidative stress, and aging. Free Radic Biol Med. 2000;29(3–4):222–30. DOI: 10.1016/s0891-5849(00)00317-8
3. Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. Eur J Med Chem. 2015;5(97):55–74. DOI: 10.1016/j.ejmech.2015.04.040
4. He L, He T, Farrar S, Ji L, Liu T, Ma X. Antioxidants Maintain Cellular Redox Homeostasis by Elimination of Reactive Oxygen Species. Cell Physiol Biochem. 2017;44(2):532–53. DOI: 10.1159/000485089
5. Zerres S, Stahl W. Carotenoids in human skin. Biochim Biophys Acta Mol Cell Biol Lipids. 2020;1865(11):158588. DOI: 10.1016/j.bbalip.2019.158588
6. Agati G, Azzarello E, Pollastri S, Tattini M. Flavonoids as antioxidants in plants: location and functional significance. Plant Sci. 2012;196:67–76. DOI: 10.1016/j.plantsci.2012.07.014
7. Grabowska M, Wawrzyniak D, Rolle K, Chomczynski P, Oziewicz S, Jurga S, Barciszewski J. Let food be your medicine: nutraceutical properties of lycopene. Food Funct. 2019;10(6):3090–102. DOI: 10.1039/c9fo00580c
8. Bhat I, Mamatha BS. Genetic factors involved in modulating lutein bioavailability. Nutr Res. 2021;91:36–43. DOI: 10.1016/j.nutres.2021.04.007
9. Berger MM. Can oxidative damage be treated nutritionally? Clin Nutr. 2005;24(2):172–83. DOI: 10.1016/j.clnu.2004.10.003
10. Lopez-Alarcon C, Denicola A. Evaluating the antioxidant capacity of natural products: a review on chemical and cellular-based assays. Anal Chim Acta. 2013;6(763):1–10. DOI: 10.1016/j.aca.2012.11.051
11. Kattoor AJ, Pothineni NVK, Palagiri D, Mehta JL. Oxidative Stress in Atherosclerosis. Curr Atheroscler Rep. 2017;19 (11):42. DOI: 10.1007/s11883-017-0678-6
12. Betteridge DJ. What is oxidative stress? Metabolism. 2000;49:3–8. DOI: 10.1016/s0026-0495(00)80077-3
13. Senoner T, Dichtl W. Oxidative Stress in Cardiovascular Diseases: Still a Therapeutic Target? Nutrients. 2019;11(9):2090. DOI: 10.3390/nu11092090
14. Bogdanov MV, Vorontsova NL, Matveeva VG, Golovkin AS, Larionov MV, Grigoriev EV. Dynamics of

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-3-219-230

oxidative stress and endointoxication in coronary sinus and peripheral blood from patients coronary artery disease undergoing coronary artery bypass grafting. Complex Issues of Cardiovascular Diseases. 2013;(4):65–70. DOI: 10.17802/2306-1278-2013-4-65-70

15. Kura B, Szeiffova Bacova B, Kalocayova B, Sykora M, Slezak J. Oxidative Stress-Responsive MicroRNAs in Heart Injury. Int J Mol Sci. 2020;21(1):358. DOI: 10.3390/ijms21010358
16. Sponga S, Bonetti A, Ferrara V, Beltrami AP, Isola M, Vendramin I, Finato N, Ortolani F, Livi U. Preservation by cold storage vs ex vivo normothermic perfusion of marginal donor hearts: clinical, histopathologic, and ultrastructural features. J Heart Lung Transplant. 2020;39(12):1408–16. DOI: 10.1016/j.healun.2020.08.021
17. Liberman EA, Topaly VP, Tsofina LM, Jasaitis AA, Skulachev VP. Mechanism of coupling of oxidative phosphorylation and the membrane potential of mitochondria. Nature. 1969;222 (5198):1076–8. DOI: 10.1038/2221076a0
18. Skulachev VP. Cationic antioxidants as a powerful tool against mitochondrial oxidative stress. Biochem Biophys Res Commun. 2013;441(2):275–9. DOI: 10.1016/j.bbrc.2013.10.063
19. Skulachev MV, Antonenko YN, Anisimov VN, Chernyak BV, Cherepanov DA, Chistyakov VA, Egorov MV, Kolosova NG, Korshunova GA, Lyamzaev KG, Plotnikov EY, Roginsky VA, Savchenko AY, Severina II, Severin FF, Shkurat TP, Tashlitsky VN, Shidlovsky KM, Vyssokikh MY, Zamyatnin AA Jr, Zorov DB, Skulachev VP. Mitochondrial-targeted plastoquinone derivatives. Effect on senescence and acute age-related pathologies. Curr Drug Targets. 2011;12(6):800–26. DOI: 10.2174/138945011795528859
20. Antonenko YN, Roginsky VA, Pashkovskaya AA, Rokitskaya TI, Kotova EA, Zaspa AA, Chernyak BV, Skulachev VP. Protective effects of mitochondria-targeted antioxidant SkQ in aqueous and lipid membrane environments. J Membr Biol. 2008;222(3):141–9. DOI: 10.1007/s00232-008-9108-6
21. Anisimov VN, Egorov MV, Krasilshchikova MS, Lyamzaev KG, Manskikh VN, Moshkin MP, Novikov EA, Popovich IG, Rogovin KA, Shabalina IG, Shekarova ON, Skulachev MV, Titova TV, Vygodin VA, Vyssokikh MY, Yurova MN, Zabezhinsky MA, Skulachev VP. Effects of the mitochondria-targeted antioxidant SkQ1 on lifespan of rodents. Aging (Albany NY). 2011;3(11): 1110–9. DOI: 10.18632/aging.100404
22. Mizobuti DS, Fogaça AR, Moraes FDSR, Moraes LHR, Mancio RD, Hermes TA, Macedo AB, Valduga AH, de Lourenco CC, Pereira ECL, Minatel E. Coenzyme Q10 supplementation acts as antioxidant on dystrophic muscle cells. Cell Stress Chaperones. 2019;24(6):1175–85. DOI: 10.1007/s12192-019-01039-2
23. Bayrak S, Aktaş S, Altun Z, Çakir Y, Tütüncü M, Kum Özşengezer S, Yilmaz O, Olgun N. Antioxidant effect of acetyl-l-carnitine against cisplatin-induced cardiotoxicity. J Int Med Res. 2020;48(8):300060520951393. DOI: 10.1177/0300060520951393
24. Förstermann U, Xia N, Li H. Roles of vascular oxidative stress and nitric oxide in the pathogenesis of atherosclerosis. Circ Res. 2017;120(4):713–35. DOI: 10.1161/CIRCRESAHA.116.309326
25. Esterbauer H, Eckl P, Ortner A. Possible mutagens derived from lipids and lipid precursors. Mutat Res. 1990;238(3):223–33. DOI: 10.1016/0165-1110(90)90014-3
26. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med. 1991;11(1):81–128. DOI: 10.1016/0891-5849(91)90192-6
27. Weismann D, Binder CJ. The innate immune response to products of phospholipid peroxidation. Biochim Biophys Acta. 2012;1818(10):2465–75. DOI: 10.1016/j.bbamem.2012.01.018
28. Liebetrau C, Nef HM, Dörr O, Gaede L, Hoffmann J, Hahnel A, Rolf A, Troidl C, Lackner KJ, Keller T, Hamm CW, Möllmann H. Release kinetics of early ischaemic biomarkers in a clinical model of acute myocardial infarction. Heart. 2014;100:652–7. DOI: 10.1136/heartjnl-2013-305253
29. Vupputuri A, Sekhar S, Krishnan S, Venugopal K, Natarajan KU. Heart-type fatty acid-binding protein (H-FABP) as an early diagnostic biomarker in patients with acute chest pain. Indian Heart J. 2015;67(6):538–42. DOI: 10.1016/j.ihj.2015.06.035
30. Collet JP, Thiele H, Barbato E, Barthelemy O, Bauersachs J, Bhatt DL, Dendale P, Dorobantu M, Edvardsen T, Folliguet T, Gale CP, Gilard M, Jobs A, Juni P, Lambrinou E, Lewis BS, Mehilli J, Meliga E, Merkely B, Mueller C, Roffi M, Rutten FH, Sibbing D, Siontis GCM; ESC Scientific Document Group. 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. Eur Heart J. 2021;42(14):1289–367. DOI: 10.1093/eurheartj/ehaa575
31. Li K, Li Y, Shelton JM, Richardson JA, Spencer E, Chen ZJ, Wang, X, Williams RS. Cytochrome c deficiency causes embryonic lethality and attenuates stress-induced apoptosis. Cell. 2000;101(4):389–99. DOI: 10.1016/s0092-8674(00)80849-1
32. Kulikov AV, Shilov ES, Mufazalov IA, Gogvadze V, Nedospasov SA, Zhivotovsky B. Cytochrome c: the Achilles' heel in apoptosis. Cell Mol Life Sci. 2012;69(11):1787–97. DOI: 10.1007/s00018-011-0895-z
33. Strobel HW, Dignam JD, Gum JR. NADPH cytochrome P-450 reductase and its role in the mixed function oxidase reaction. Pharmacol Ther. 1980;8(3):525–37. DOI: 10.1016/0163-7258(80)90075-3
34. Olejnickova V, Novakova M, Provaznik I. Isolated heart models: cardiovascular system studies and technological advances. Med Biol Eng Comput. 2015;53(7):669–78. DOI: 10.1007/s11517-015-1270-2
35. Senokosova EA, Krutitskiy SS, Velikanova EA, Tsepokina AV, Kuzmina AA, Tretjak VM, Denisova SV, Gruzdeva OV, Antonova LV, Grigoriev EV. Ability of N(2)-L-alanyl-L-glutamine to restore the function of ischemic myocard. Complex Issues of Cardiovascular Diseases. 2017;(1):44–50. DOI: 10.17802/2306-1278-2017-1-44-50
36. Firsov AM, Kotova EA, Orlov VN, Antonenko YN, Skulachev VP. A mitochondria-targeted antioxidant can inhibit peroxidase activity of cytochrome c by detachment of the protein from liposomes. FEBS Lett. 2016;590(17):2836–43. DOI: 10.1002/1873-3468.12319
37. Bakeeva LE, Barskov IV, Egorov MV, Isaev NK, Kapelko VI, Kazachenko AV, Kirpatovsky VI, Kozlovsky SV, Lakomkin VL, Levina SB, Pisarenko OI, Plotnikov EY, Saprunova VB, Serebryakova LI, Skulachev MV, Stelmashook EV, Studneva IM, Tskitishvili OV, Vasilyeva AK, Victorov IV, Zorov DB, Skulachev VP. Mitochondria-targeted

plastoquinone derivatives as tools to interrupt execution of the aging program. 2. Treatment of some ROS- and age-related diseases (heart arrhythmia, heart infarctions, kidney ischemia, and stroke). Biochemistry (Mosc). 2008;73(12):1288–99. DOI: 10.1134/s000629790812002x

38. Hamed M, Logan A, Gruszczyk AV, Beach TE, James AM, Dare AJ, Barlow A, Martin J, Georgakopoulos N, Gane AM, Crick K, Fouto D, Fear C, Thiru S, Dolezalova N, Ferdinand JR, Clatworthy MR, Hosgood SA, Nicholson ML, Murphy MP, Saeb-Parsy K. Mitochondria-targeted antioxidant MitoQ ameliorates ischaemia-reperfusion injury in kidney transplantation models. Br J Surg. 2021;108(9):1072–81. DOI: 10.1093/bjs/znab108

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