UDC 577.353.9.616.74 doi: https://doi.org/10.15407/ubj90.06.070

INFLUENCE OF C_{60} FULLERENE ON THE ISCHEMIA-REPERFUSION INJURY IN THE SKELETAL MUSCLE OF RAT LIMB: MECHANOKINETIC AND BIOCHEMICAL ANALYSIS

D. O. ZAVODOVSKYI[1]✉, S. Yu. ZAY[2], T. Yu. MATVIENKO[1], Yu. I. PRYLUTSKYY[1], N. Y. NURISHCHENKO[1], S. S. PARADIZOVA[3], L. L. BEZUH[3], U. RITTER[4], P. SCHARFF[4]

[1]*ESC Institute of Biology and Medicine, Taras Shevchenko National University of Kyiv, Ukraine;*
[2]*Lesya Ukrainka Eastern European National University, Lutsk, Ukraine;*
[3]*SI The Territorial Medical Association of the Ministry of Internal Affairs of Ukraine in Kyiv;*
[4]*Institute of Chemistry and Biotechnology, Technical University of Ilmenau, Ilmenau, Germany;*
✉*e-mail: Lab@univ.kiev.ua*

Influence of the pristine C_{60} fullerene aqueous colloidal solution (C_{60}FAS) on the ischemia-reperfusion injury in the skeletal muscle of rat limb was studied. Skeletal muscle damage effects were induced by 3 h lasting vascular ischemia. The impact of C_{60}FAS was studied after its intramuscular injection immediately after 1 h of reperfusion at different doses, namely: 1, 2 and 3 mg/kg of body weight. Changes in the mechanokinetic parameters of ischemic skeletal muscle contraction at different modes of functioning and biochemical parameters of blood were used as markers of ischemic injury, and analyzed in detail under action of C_{60}FAS. The obtained results indicate on great promise of use of C_{60}FAS to reduce the consequences of ischemic muscle trauma.

K e y w o r d s: C_{60} fullerene, skeletal muscle, ischemia-reperfusion injury, mechanokinetic and biochemical analysis.

Ischemic injuries constitute more than 35% among various injury-induced pathologies that develop in skeletal muscles [1]. They are a major cause of postoperative complications, and can lead to amputations of limbs and even mortality after acute arterial occlusion [2]. Effects of skeletal muscle ischemia depend on the degree of microvasculature damage, level of tissue hypoxia, amount and value of the products of metabolism, ratio of extracellular and intracellular concentrations of Na^+, K^+, H^+, mechanical damage to muscles, etc. [2, 3]. Free radicals that are rapidly formed in ischemic muscle injury, are another major threat to the organism of ischemic patients [4]. Interaction of hydroxyl radical with hydrogen atoms of methyl groups of polyunsaturated fatty acids is considered one of the major mechanisms of tissue damage by free radicals. This process initiates lipid peroxidation (LPO) in plasma membrane, which in turn leads to increased permeability of cell membranes. In this regard, use of antioxidants is considered promising direction in the treatment of muscle damage [4-7].

Some nanomaterials show strong antioxidant properties and can be used for the treatment of ischemia injury of different organs [8]. So, C_{60} fullerenes behave as “free radical scavengers” and demonstrate antioxidant activity in micromolar concentrations, eliminating both H_2O_2 and superoxide anions [9, 10]. Due to its nearly spherical shape and nanosize, water-soluble C_{60} fullerene is capable to penetrate plasma membrane of mammalian cells [11-13]. *In vitro* and *in vivo* studies of water-soluble pristine C_{60} fullerenes had found that these compounds do not possess acute toxicity towards cells and tissues of human organism [14-17]. Thus, the main advantage of use of C_{60} fullerenes as powerful anti-

oxidants is their ability to rapidly accumulate inside the cells in the mitochondria and other organelles, responsible for generation of toxic free radicals under pathological conditions [11, 18, 19].

Thus, the purpose of this study was to evaluate the impact of water-soluble pristine C_{60} fullerenes on the mechanokinetic and biochemical parametersof rat soleus muscle functioning at the ischemia-reperfusion injury.

Materials and Methods

A highly stable pristine C_{60} fullerene aqueous colloidal solution (C_{60}FAS) was prepared according to protocol [20, 21]. The final concentration of C_{60} fullerenes in the prepared C_{60}FAS sample was 0.15 mg/ml.

The state of C_{60} fullerene particles (aggregation) in aqueous solution was monitored using atomic force microscopy (AFM; NT-MDT, Russia). The samples were deposited by precipitation from C_{60}FAS droplet onto a cleaved mica surface. Measurements were performed after complete evaporation of the solvent. The sample visualization was performed in semi-contact (tapping) mode.

The study was conducted on white male rats of the Wistar line weighing 170 ± 5 g. The animals were kept under standard conditions in the vivarium of the ESC Institute of Biology and Medicine, Taras Shevchenko National University of Kyiv. Animals had free access to food and water. All experiments were conducted in accordance with the international principles of the European Convention for protection of vertebrate animals under a control of the Bio-Ethics Committee of the abovementioned institution.

All experimental animals were divided into five groups: intact group (n = 10), control group (animals after ischemia without injection of C_{60}FAS; n = 10), and three experimental groups: animals after ischemia with different doses of C_{60}FAS intramuscular injection immediately after reperfusion – 1 mg/kg (n = 10), 2 mg/kg (n = 10), and 3 mg/kg (n = 10).

Under severe anesthesia (ketamine: 100 mg/kg, Pfizer, USA), the animals were tracheotomized and connected to the lung ventilator. Musculus soleus was isolated in the area of the popliteal hollow, cut proximally and attached to the force sensors. The animal then was fixed in a stereotactic machine with a system of rigid fixation of the head, pelvis and extremities. The innervating musculus soleus nerve was fixed on a bipolar platinum wire electrode for further electrical stimulation. The edges of the skin on the hind limbs around the incision were sewn to the machine armature, and basins with muscle and nerve that were formed, were filled with vaseline oil. During the operation and the experiment by itself, the heart rate was monitored.

In order to get muscle ischemia, the branch of the femoral artery, which provided blood to the experimental muscle for 3 h, was ligated.

Before stimulation of the ventral roots of the spinal cord, the muscle was attached to an external load that could not stretch it due to a one-sided mechanical limiter that allowed only a muscle shortening.

The force of the skeletal muscle contraction was measured with the usage of unique strain gauges in 1 h after ischemia-reperfusion. Programmable signal generators of a special form were used to generate stimulating signals. The contraction of muscles was induced by the stimulation of the nervus ischiadicus. The electric pulses were 2 ms duration, formed by a pulse generator. Control of the external load on the muscle was carried out with the help of the original system of mechanical stimulators [22].

As muscle contraction markers, the time of reducing the maximum contraction force to 25% and 50% levels were measured. As a marker of quantitative changes in muscle activity, the integrated muscle power was calculated (the total area under the force curve). The force response of musculus soleus in the control group was accepted as 100%.

The content of creatinine, creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) enzymes in the blood of experimental animals, as the markers of ischemic injury of the skeletal muscle, was determined using the standard clinical equipment (Selectra Pro XL EliTechGroup, France).

The mechanokinetic and biochemical data are expressed as the mean±SD for each group. The differences among experimental groups were detected by one-way ANOVA followed by Bonferroni's multiple comparison test. Values of $P < 0.05$ were considered significant.

Results and Discussion

AFM measurements. It is established that the size of C_{60} fullerene aggregates in aqueous solution strongly correlates with their biological properties. Thus, the apoptosis in macrophages induced by aqueous C_{60} fullerene aggregates, leads to changes in the mitochondrial membrane potential [23], the aggregated forms of C_{60} fullerene may effectively

bind to biopolymers [24], and antibacterial activity of C_{60} fullerene is tightly connected with its ability to undergo aggregation [25]. Depending on the size C_{60} fullerene particles can penetrate through plasma membrane inside the cell through endocytosis or passive diffusion [11, 13, 26, 27] or be adsorbed on the surface of the membrane [12]. Therefore, the size of C_{60} fullerene particles in aqueous solution has a major influence on their biological properties.

Fig. 1 shows the AFM image of surface area covered by C_{60} fullerenes (~0.7 nm) and their aggregates with size up to 100 nm. Thus, C_{60}FAS is a polydisperse colloidal nanosystem. This result is in a good agreement with our previous probe microscopic data which directly correlate with C_{60} fullerene bioactivity [28, 29].

Mechanokinetic analysis. One of the main parameters of muscle pathology development is measurement of the force myotonic response. The contractile dynamics of the muscle is determined by specific mechanisms of motoneuronic pools interaction and activation of actin and myosin myofilaments interaction. Changes in the elastic properties of muscle fibers, tendon elements and connective tissue determine the force response of contractive elastic component, which is a consequence of pathological process development.

Several basic biomechanical parameters were investigated during analysis of musculus soleus myotonic response by using the frequency-modulated stimulating signal. These parameters are universally recognizable markers for the presence of particular link dysfunctions in the "stimulation-response" chain as a neuromuscular drug, as well as the general state of the organism.

Determination of the muscle contraction force generation level. This marker is a general indicator of the reduction (during the pathology development) of the maximum possible force response. Its change can be related either to a violation in the neuronal component, or to the myotic components of the pathology that is studied.

Determination of the muscle contraction integrated power. The integrated power (calculated area that is bounded by the force curve: $S = \int_{t_1}^{t_2} F dt$, $F = \frac{Pt}{l}$, where P is a muscle power, and l = const is its length) is an indicator of the overall capacity of the muscle during application of the stimulation pools. Its analysis allows assessing the level of muscle activity formation in the "force-external load" equilibrium system that is a physiological analogue of the working capacity of the muscular system in general.

Determination of the temporal characteristics of muscle contraction in the examined pathology development. Evaluation of muscle contraction

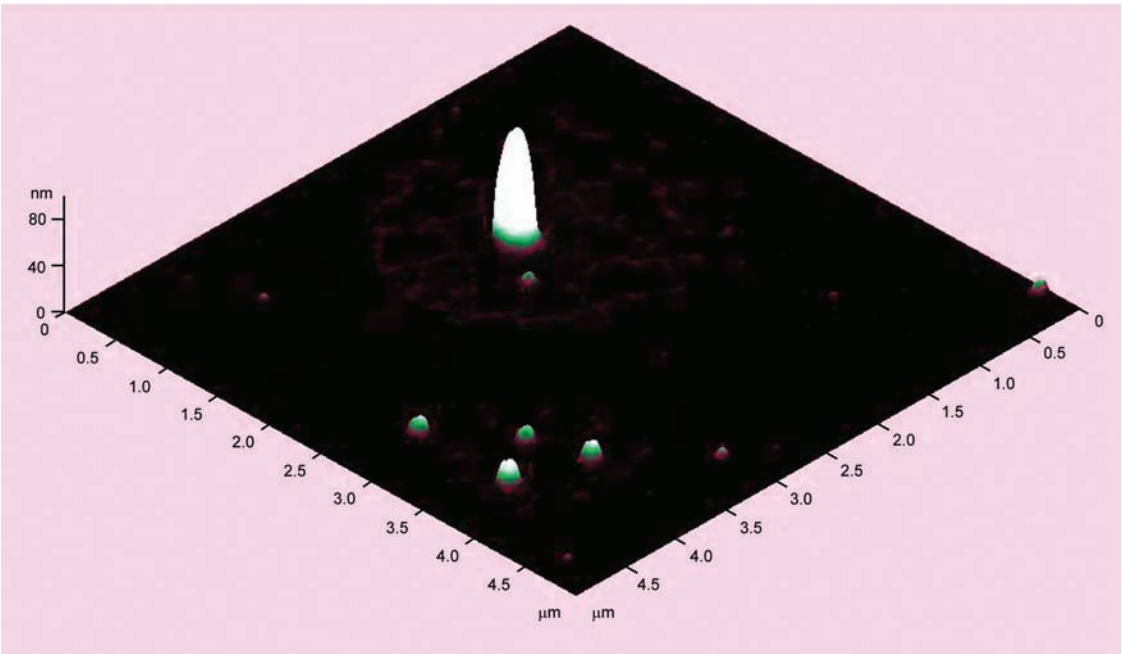

Fig. 1. AFM image (tapping mode) of C_{60} fullerene nanoparticles on the mica surface (concentration 0.15 mg/ml)

development was carried out by calculating time indexes (time of achievement and retention of the attained state of the contractile process) when 25 and 50% of force levels responses were obtained after stimulation.

The curves of contraction forces generation (Fig. 2), the value of the achievement time by the force of 25 and 50% of its initial level (Fig. 3) and the relative value of the integrated power (Fig. 4) of ischemic musculus soleus as a response to stimulation at frequencies of 1 and 2 Hz clearly show a tendency to reduce the maximal force response, the time of achievement by contraction force its 25 and 50% levels and integrated power of the active muscle throughout the experiment compared to norm (the intact group; data not shown). It should be noted that the maximum changes of the muscles force response occur in the first minutes of force parameters registration (Fig. 2).

After 150 s of muscle activation the force response does not exceed 18% and 12% of the initial parameters at the frequency of 1 and 2 Hz, respectively. The time to reach 25% and 50% level of the initial force was 127 and 145 s with the frequency of

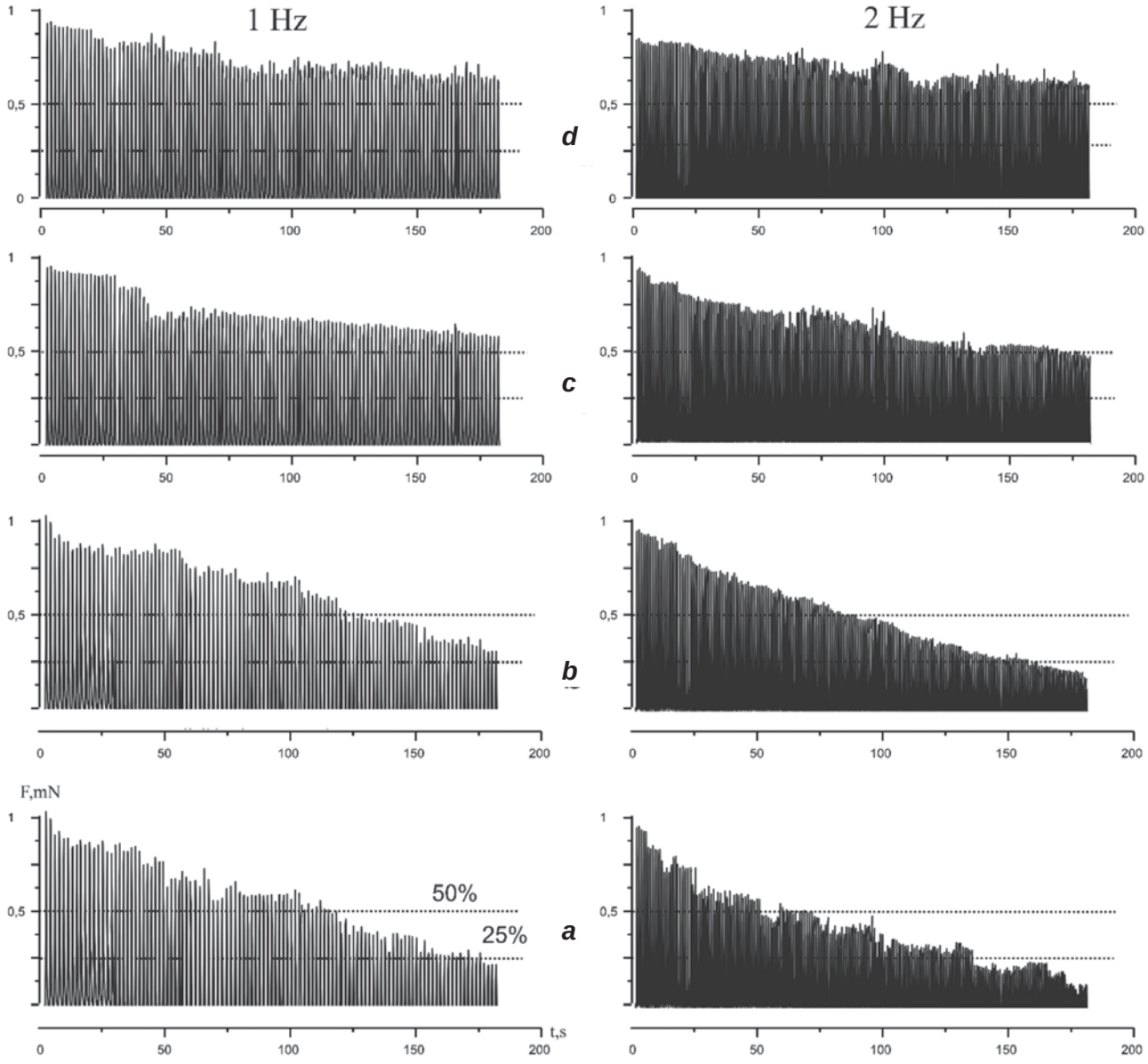

Fig. 2. Curves of ischemic musculus soleus force contraction generation during stimulation with 1 and 2 Hz frequencies and 180 s duration: ***a*** *– ischemic muscle contraction without injection of* C_{60}*FAS (control);* ***b***, ***c*** *and* ***d*** *– ischemic muscle contraction after intramuscular injection of 1, 2 and 3 mg/kg* C_{60}*FAS, respectively*

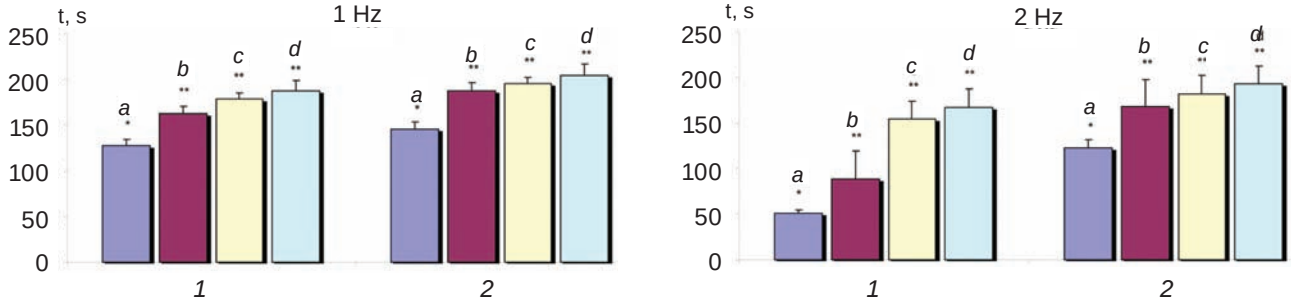

*Fig. 3. Time of achievement by ischemic musculus soleus contraction force of 25% (**1**) and 50% (**2**) level from the initial value during stimulation with 1 and 2 Hz frequencies and 180 s duration: **a** – ischemic muscle contraction without injection of C_{60}FAS (control); **b**, **c** and **d** – ischemic muscle contraction after intramuscular injection of 1, 2 and 3 mg/kg C_{60}FAS, respectively. *P < 0.05; **P < 0.05 compared with control*

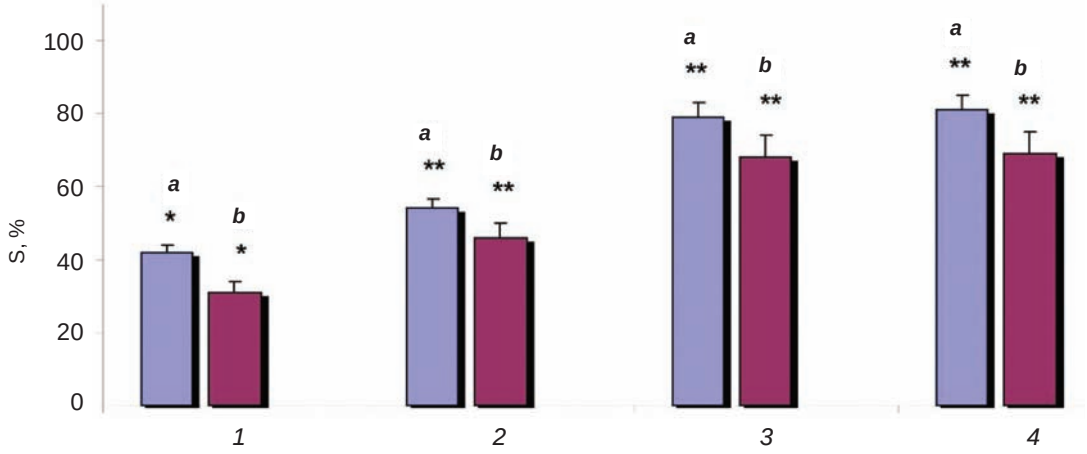

*Fig. 4. Integrated power value (as a percentage of 100% control) of successive single ischemic musculus soleus contractions during stimulation with 1 Hz (**a**) and 2 Hz (**b**) frequencies and 180 s duration: 1 – ischemic muscle contraction without injection of C_{60}FAS (control); **2**, **3** and **4** – ischemic muscle contraction after intramuscular injection of 1, 2 and 3 mg/kg C_{60}FAS, respectively. *P < 0.05; **P < 0.05 compared with control*

stimulation 1 Hz and 51 and 123 s with the frequency of stimulation 2 Hz (Fig. 3, *a*). The data show the presence of severe pathological changes in muscle dynamics associated with ischemic dysfunction of muscle tissue.

In the case of a muscle force response study, the protective effect of C_{60}FAS strongly manifested itself with an increase in the dose of injection (Fig. 2, *b*, *c*, *d*) compared to control (Fig. 2, *a*). The time to reach 25% and 50% of primary force with the C_{60}FAS application in a dose of 1 mg/kg was 162 and 187 s with the stimulation frequency of 1 Hz and 89 and 168 s with the stimulation frequency of 2 Hz (Fig. 3, *b*). Increasing the dosage of C_{60}FAS in 2-3 times (2-3 mg/kg) increased the time to achieve a 50% force level only by 5-6% when the stimulation was 1 Hz and 6-8% during stimulation of 2 Hz compared to dose of 1 mg/kg (Fig. 3, *c*, *d*). Thus, the C_{60}FAS dose of 1 mg/kg revealed an optimal ratio of "dose-effect".

The magnitude of the integrated power in the case of using 1 mg/kg C_{60}FAS increased by 16% during stimulation at 1 Hz frequency and by 21% during stimulation at 2 Hz frequency compared to the control (Fig. 4). Increasing the dose of C_{60}FAS in 2-3 times increases the integrated power by 16-22% with stimulation at 1 Hz frequency and by 25-31% during stimulation at 2 Hz frequency compared to the 1 mg/kg dose of C_{60}FAS. It should also be mentioned that the difference in the studied parameter in the case of 2 and 3 mg/kg C FAS doses is insignificant amongst themselves (within the limits of statistical error; Fig. 4).

While studying the muscle contractile properties, is it important not only to know the current values of the force response and the intensity of muscle activation, but also their temporal dynamics. A consequence of the neuromuscular complex development dysfunction is the necessity for motoneurons to generate the sufficiently powerful dynamic stimulation components to resume the error-free functioning of the muscular system. Thus, at the same levels of the efferent command, not only does an increase of the preceding dynamic component duration slow down the transition to a new equilibrium force, but also leads to a decrease in the maximum force response. A series of 10 consecutive stimulations of an ischemic damaged muscle with duration 6 s, 50 Hz frequency and 5 min relaxation was used to reveal such effect on the studied areas of the dynamic process (Fig. 5).

As it could be seen from Fig. 4, *a*, the contraction force in ischemic muscles at the first stimulation pool decreased to 50% relatively to the norm (intact group) and decreased to 11% to the 10th contractile act. The protective effect of C_{60}FAS was observed even at the minimum applied dose of the

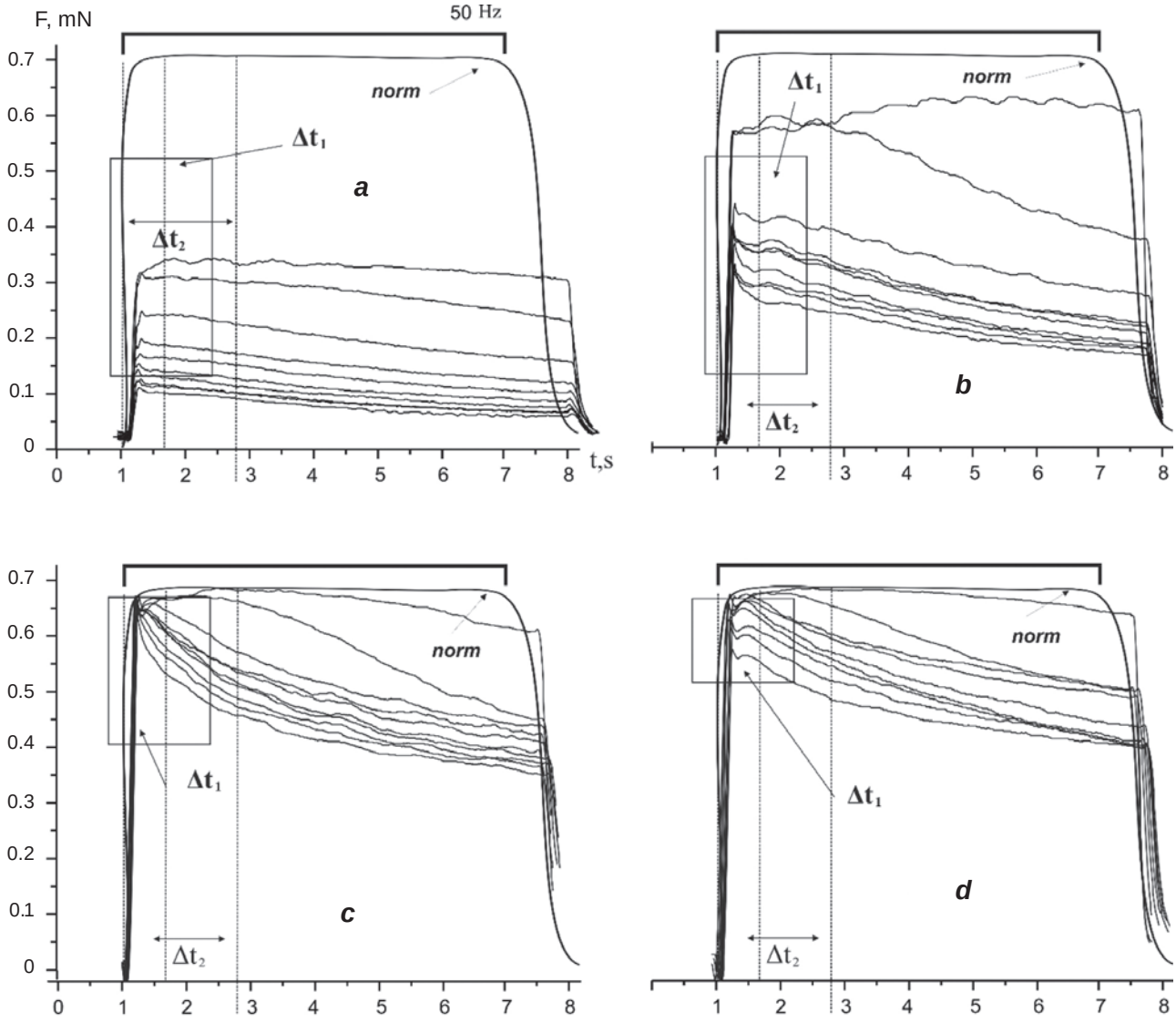

Fig. 5. Curves of ischemic musculus soleus tetanic force contraction generation caused by 10 successive 50 Hz stimuli with duration of 6 s and 5 min relaxation: ***a*** *– ischemic muscle contraction without injection of C_{60}FAS (control);* ***b***, ***c*** *and* ***d*** *– ischemic muscle contraction after intramuscular injection of 1, 2 and 3 mg/kg C_{60}FAS, respectively; norm – muscle contraction in the intact group; Δt_1 and Δt_2 – time of reaching and maintaining the maximum level of muscle contraction*

drug – 1 mg/kg (Fig. 5, *b*). The maximum force response relatively to the norm at the 10th contraction increased by 55% at C_{60}FAS dose of 1 mg/kg and by 83 and 87% at C_{60}FAS doses of 2 and 3 mg/kg, respectively (Fig. 5, *b*, *c*, d). The increase in integrated muscle power in the application of C_{60}FAS was similar: by 48% at the 10th contraction at 1 mg/kg C_{60}FAS dose and by 77 and 80% at 2 and 3 mg/kg C_{60}FAS dose, respectively.

As in the previous experiments, significant dose dependence was observed only between 1 and 2 mg/kg C_{60}FAS doses: an increase of C_{60}FAS dose from 2 to 3 mg/kg resulted in a slight improvement in muscle contraction parameters that did not exceed 10%.

The previously discovered high correlation between the duration of ischemia and the viability of muscle fiber [30], could be one of the main factors for reducing the maximum force response, not only because of the decrease in viable muscle fibers, but also due to an increase in muscle stiffness because of the increase in collagen structures in it. During ischemia lasting for 3 h both necrotic muscular changes and nervous degeneration are observed, and the amount of necrosis in the muscle tissue can reach up to 60% [31]. Thus, the use of C_{60}FAS in ischemic injury may have a pronounced positive normalizing effect, mainly at an early stage of development of such pathology (~1-2 h).

Biochemical analysis. At the biochemical level, ischemic damage of the muscle tissue is a sequence of biochemical reactions initiated by hypoxia after several minutes of ischemia, which, regardless of etiological features, is the result of insufficient blood muscle supply [1]. The death of the majority of cells occurs due to the activation of chemicals produced during and after ischemia and can continue for several days even after the restoration of normal blood flow. Today, it is known that after 2 h of skeletal muscle ischemia and subsequent reperfusion, the concentration of ATP is significantly reduced at the same time with a significant increase in the amount of lactate – from 25 to 114 mmol/kg of dry weight. After 3 h of ischemia, the intramuscular pool of ATP is about 5% of the baseline level, while the glycogen pool is exhausted by 88% [34]. From the functional point of view, these values indicate that a significant amount of high-energy phosphate compounds is consumed by the damaged ischemic-muscle cell for the homeostasis support, especially during the first hour of ischemia. As a result, metabolic disorders lead to a dramatic increase in fatigue of ischemic muscle.

Besides the danger caused by the release of the above-mentioned macromolecules to the ischemic muscle tissue and the entire body, free radicals that are intensively formed by ischemic injury, possess even higher threat to the organism. Free radicals, in particular superoxide anions and hydroxyl radicals, are the main pathogenic factors in the process of ischemic-reperfusion damage of muscle tissue. They initiate the LPO and direct inhibition of mitochondrial enzymes in the respiratory chain, inhibit the ATPase activity, inactivate the glyceraldehyde-3-phosphate dehydrogenase, sodium channels in cell membrane and launch a number of other pathophysiological processes. All this indicates on the extremely high risk of reperfusion damage to the skeletal muscle. Thus, it is not surprising that reperfusion injury, observed after acute arterial occlusion, is the cause of the most severe pathologies and even mortality [2]. Therefore, the use of biocompatible water-soluble C_{60} fullerenes, which have powerful antioxidant properties [35, 36] may be extremely promising for treatment of various pathologies, connected to oxidative stress [4-8, 37, 38]. It is important to note that C_{60} fullerenes reduce the production of reactive oxygen species in muscle tissue, which is confirmed by physical (EPR spectroscopy and spin traps) and biochemical methods [4, 5, 7, 8].

All of the above-analyzed factors allow us to consider ischemic-reperfusion injury as a complex pathophysiological process that affects a large number of metabolic links directly at the place of pathology development, and generally in the body. For evaluation of ischemic-reperfusion injury, several markers were selected which are specific for muscle damage and can be measured by standard blood biochemical assays – creatinine, CPK and LDH enzymes.

Creatinine is a breakdown product of creatine phosphate, that serves as a rapidly mobilizable reserve of high-energy phosphates in skeletal muscle. During ischemic-reperfusion skeletal muscle injury creatinine is released from damaged muscle tissue to the blood, while its elimination by the kidneys is decreased due to renal insufficiency. 3-fold increase in creatinine level after 3 h of muscle ischemia in comparison with the intact group (norm; Fig. 6) indicates the presence of severe mechanical destruction of muscle fibers in the experimental limb. Reduction of this index by 18, 40 and 47% after injection of

animals with 1, 2 and 3 mg/kg of the C_{60}FAS doses clearly demonstrates its protective effect (Fig. 6).

CPK is the enzyme that catalyzes the transfer of a phosphate group from ATP to a creatine molecule that results in a high-energy creatine phosphate compound formation that is used with the body as an energy substance during increased physical activity. When muscles are damaged, release of this enzyme from the cells into the blood is observed. In our opinion, a 2-fold increase of the CPK fraction after 3 h of muscle ischemia (norm; Fig. 6) is the result of pathological destruction of the myocyte walls with partial release of intramyocytic enzymes in the extracellular space, while the decrease in the CPK concentration by 20-35% is due to the normalizing effect of C_{60}FAS (Fig. 6).

LDH is a zinc intracellular enzyme that catalyzes the oxidation of lactic acid (the final product of glucose metabolism in cells in the absence of oxygen, for example, during prolonged physical activity) to pyruvate with the formation of NADH and is present in almost all cells of the body. In diseases that are accompanied by tissue damage and cell destruction, LDH activity in the blood increases. In conducted studies, LDH level increased after 3 h of musculus soleus ischemic damage 1.5 fold compared to the intact group (norm; Fig. 6), which is consistent with the literature data [30, 31]. On the contrary, C_{60}FAS efficiently inhibited LDH release in dose-dependent manner – by 15% after 1 mg/kg dose and 25% after 3 mg/kg dose (Fig. 6).

Thus, changes in the examined biochemical markers of ischemia for different injected doses of C_{60}FAS indicate on its overall positive normalizing effect, in contrast to the mechanokinetic parameters, when the optimal positive changes were observed already at the 1 mg/kg C_{60}FAS dose and varied by 10% level after each subsequent increase in C_{60}FAS concentration.

Despite development of novel experimental approaches for analysis of the neuromuscular regulation at the microlevel, there is still no alternative to traditional electrophysiological models using neuromuscular preparations *in vivo*. Such studies should be conducted with the aim of simultaneous monitoring of various biomechanical parameters with different amplitude-time intervals and a labile system of external stimulation. In the framework of such approach, it was established that the therapeutic C_{60}FAS injection can significantly reduce the negative pathological changes in the mechanokinetic parameters of the skeletal muscle functioning after 3 h

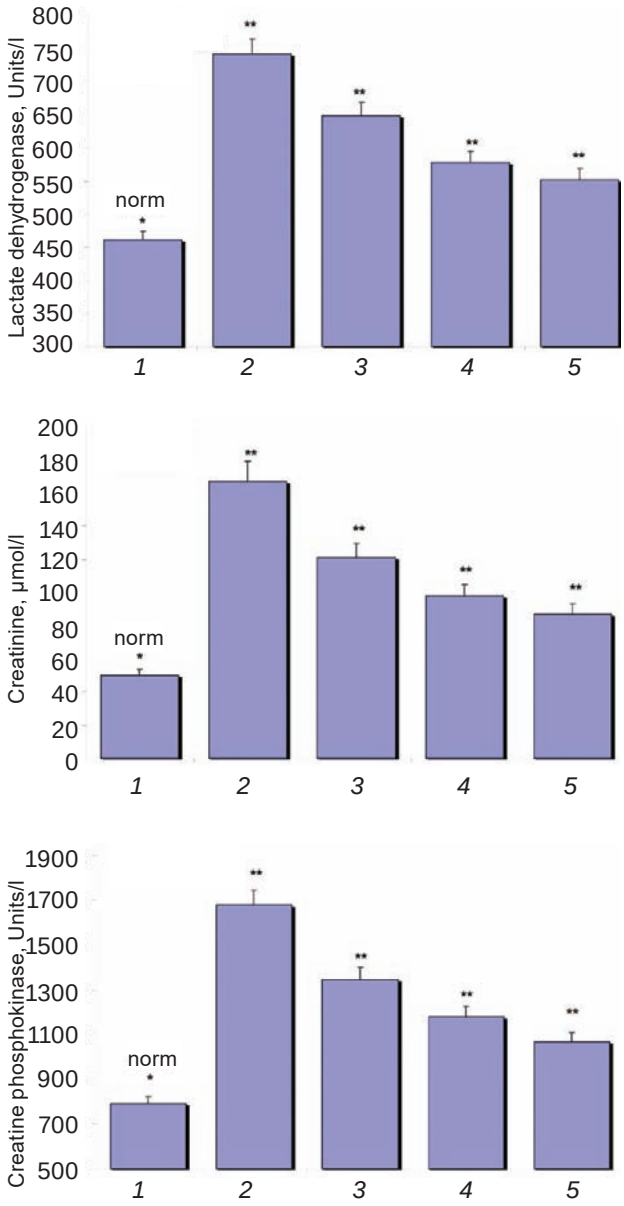

*Fig. 6. Levels of creatinine, creatine phosphokinase and lactate dehydrogenase in the blood of ischemic rats after musculus soleus tetanic contractions caused by 10 successive 50 Hz stimuli with duration of 6 s and 5 min relaxation: norm – intact group; 2 – control group; 3, 4 and 5 – ischemic muscle contraction after intramuscular injection of 1, 2 and 3 mg/kg C_{60}FAS, respectively *P < 0.05; **P < 0.05 compared to the intact group*

of ischemia and 1 h of reperfusion. Positive therapeutic changes were recorded for both tetanic and single muscle contractions. For ischemic-reperfusion injury, a significant increase of creatinine, CPK and LDH in blood was observed, while the injection of C_{60}FAS significantly reduced these markers of

ischemic injury. However, further 2-3-fold increase of C_{60}FAS dose led to a little increase of its efficiency, which can be explained by the property of C_{60} fullerenes to aggregate in the aqueous medium [39, 40]. This can lead to potential decrease of their biological activity [23, 25], thus we consider that the optimal C_{60}FAS dosage is 1 mg/kg of animal weight.

Consequently, taking into account the pronounced antioxidant properties of water-soluble C_{60} fullerenes and the lack of data about their acute and chronic toxicity, their use in therapy of ischemic-reperfusion damage of muscle tissue opens up new possibilities in modern nanomedicine.

Conflict of interests. The authors report no conflict of interests. The authors alone are responsible for the content and writing of the paper.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

ВПЛИВ ФУЛЕРЕНУ C_{60} НА ІШЕМІЧНО-РЕПЕРФУЗІЙНУ ТРАВМУ СКЕЛЕТНОГО М'ЯЗА КІНЦІВКИ ЩУРА: МЕХАНОКІНЕТИЧНИЙ ТА БІОХІМІЧНИЙ АНАЛІЗИ

Д. О. Заводовський[1]✉, С. Ю. Зай[2], Т. Ю. Матвієнко[1], Ю. І. Прилуцький[1], Н. Є. Нурищенко[1], С. С. Парадізова[3], Л. Л. Безух[3], У. Ріттер[4], П. Шарф[4]

[1]ННЦ «Інститут біології та медицини», Київський національний університет імені Тараса Шевченка, Україна;
[2]Східноєвропейський національний університет імені Лесі Українки, Луцьк, Україна;
[3]ДУ «Територіальне медичне об'єднання МВС України по м. Києву», Україна;
[4]Інститут хімії та біотехнології, Технічний університет Ільменау, Німеччина;
✉e-mail: Lab@univ.kiev.ua

Вивчено вплив водно-колоїдного розчину фулерену C_{60} (C_{60}FAS) на ішемічно-реперфузійну травму скелетного м'яза задньої кінцівки щура. Ушкодження скелетного м'яза були спричинені васкулярною ішемізацією тривалістю 3 год. Вплив C_{60}FAS вивчали шляхом його внутрішньом'язової ін'єкції після 1 год реперфузії за різних доз введення: 1, 2 і 3 мг/кг маси тіла тварини. Як маркери ішемічного ушкодження м'язів було використано зміни механокінетичних параметрів скорочення ішемізованого скелетного м'яза за різних режимів функціонування та біохімічні параметри крові тварин, які було детально досліджено за дії C_{60}FAS. Одержані результати вказують на перспективність використання C_{60}FAS для зменшення наслідків ішемічної травми м'яза.

Ключові слова: фулерен C_{60}, скелетний м'яз, ішемічно-реперфузійна травма, механокінетичний та біохімічний аналізи.

ВЛИЯНИЕ ФУЛЛЕРЕНА C_{60} НА ИШЕМИЧЕСКИ-РЕПЕРФУЗИОННУЮ ТРАВМУ СКЕЛЕТНОЙ МЫШЦЫ КОНЕЧНОСТИ КРЫСЫ: МЕХАНОКИНЕТИЧЕСКИЙ И БИОХИМИЧЕСКИЙ АНАЛИЗЫ

Д. А. Заводовский[1]✉, С. Ю. Зай[2], Т. Ю. Матвиенко[1], Ю. И. Прилуцкий[1], Н. Е. Нурищенко[1], С. С. Парадизова[3], Л. Л. Безух[3], У. Риттер[4], П. Шарф[4]

[1]УНЦ «Институт биологии и медицины», Киевский национальный университет имени Тараса Шевченко, Украина;
[2]Восточноевропейский национальный университет имени Леси Украинки, Луцк, Украина;
[3]ГУ «Территориальное медицинское объединение МВД Украины по г. Киеву», Украина;
[4]Институт химии и биотехнологии, Технический университет Ильменау, Германия;
✉e-mail: Lab@univ.kiev.ua

Изучено влияние водно-коллоидного раствора фуллерена C_{60} (C_{60}FAS) на ишемически-реперфузионную травму скелетной мышцы задней конечности крысы. Повреждения скелетной мышцы были вызваны васкулярной ишемией длительностью 3 ч. Влияние C_{60}FAS изучали после его внутримышечной инъекции по истечении 1 ч реперфузии при разных дозах введения: 1, 2 и 3 мг/кг массы тела животного. В качестве маркеров ишемического повреждения были использованы механокинетические параметры сокращения ишемизированной скелетной мышцы при различных режимах функционирования и биохимические параметры кро-

ви, детально изученные при воздействии C_{60}FAS. Полученные результаты указывают на перспективность использования C_{60}FAS для снижения последствий ишемической травмы мышцы.

Ключевые слова: фуллерен C_{60}, скелетная мышца, ишемически-реперфузионная травма, механокинетический и биохимический анализы.

References

1. Murdock M, Murdoch MM. Compartment syndrome: a review of the literature. *Clin Podiatr Med Surg.* 2012; 29(2): 301-310.
2. Erkut B, Özyazıcıoğlu A, Karapolat BS, Koçoğulları CU, Keles S, Ateş A, Gundogdu C, Kocak H. Effects of ascorbic Acid, alpha-tocopherol and allopurinol on ischemia-reperfusion injury in rabbit skeletal muscle: an experimental study. *Drug Target Insights.* 2007; 2: 249-258.
3. Vignaud A, Hourde C, Medja F, Agbulut O, Butler-Browne G, Ferry A. Impaired skeletal muscle repair after ischemia-reperfusion injury in mice. *J Biomed Biotechnol.* 2010; 2010: 724914.
4. Cuzzocrea S, Riley DP, Caputi AP, Salvemini D. Antioxidant therapy: a new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. *Pharmacol Rev.* 2001; 53(1): 135-159.
5. Prylutskyy YI, Vereshchaka IV, Maznycheko AV, Bulgakova NV, Gonchar OO, Kyzyma OA, Ritter U, Scharff P, Tomiak T, Nozdrenko DM, Mishchenko IV, Kostyukov AI. C_{60} fullerene as promising therapeutic agent for correcting and preventing skeletal muscle fatigue. *J Nanobiotechnology.* 2017; 15(1): 8.
6. Nozdrenko DM, Zavodovskyi DO, Matvienko TY, Zay SY, Bogutska KI, Prylutskyy YI, Ritter U, Scharff P. C_{60} fullerene as promising therapeutic agent for the prevention and correction of skeletal muscle functioning at ischemic injury. *Nanoscale Res Lett.* 2017; 12(1): 115.
7. Vereshchaka IV, Bulgakova NV, Maznychenko AV, Gonchar OO, Prylutskyy YI, Ritter U, Moska W, Tomiak T, Nozdrenko DM, Mishchenko IV, Kostyukov AI. C(60) Fullerenes Diminish Muscle Fatigue in Rats Comparable to N-acetylcysteine or β-Alanine. *Front Physiol.* 2018; 9: 517.
8. Amani H, Habibey R, Hajmiresmail SJ, Latifi S, Pazoki-Toroudi H, Akhavan O. Antioxidant nanomaterials in advanced diagnoses and treatments of ischemia reperfusion injuries. *J Mater Chem B.* 2017; 5(48): 9452-9476.
9. Wang IC, Tai LA, Lee DD, Kanakamma PP, Shen CK, Luh TY, Cheng CH, Hwang KC. C(60) and water-soluble fullerene derivatives as antioxidants against radical-initiated lipid peroxidation. *J Med Chem.* 1999; 42(22): 4614-4620.
10. Burlaka AP, Sidorik YP, Prylutska SV, Matyshevska OP, Golub OA, Prylutskyy YI, Scharff P. Catalytic system of the reactive oxygen species on the C_{60} fullerene basis. *Exp Oncol.* 2004; 26(4): 326-327.
11. Foley S, Crowley C, Smaihi M, Bonfils C, Erlanger BF, Seta P, Larroque C. Cellular localisation of a water-soluble fullerene derivative. *Biochem Biophys Res Commun.* 2002; 294(1): 116-119.
12. Schuetze C, Ritter U, Scharff P, Fernekorn U, Prylutska S, Bychko A, Rybalchenko V, Prylutskyy Yu. Interaction of N-fluorescein-5-isothiocyanate pyrrolidine-C_{60} with a bimolecular lipid model membrane. *Mater Sci Eng C.* 2011; 31(5): 1148-1150.
13. Franskevych D, Palyvoda K, Petukhov D, Prylutska S, Grynyuk I, Schuetze C, Drobot L, Matyshevska O, Ritter U. Fullerene C(60) Penetration into Leukemic Cells and Its Photoinduced Cytotoxic Effects. *Nanoscale Res Lett.* 2017; 12(1): 40.
14. Sayes CM., Fortner JD, Guo W, Lyon D, Boyd AM, Ausman KD, Tao YJ, Sitharaman B, Wilson LJ, Hughes BJ, West JL, Colvin VL. The differential cytotoxicity of water-soluble fullerenes. *Nano Lett.* 2004; 4(10): 1881-1887.
15. Prylutska SV, Matyshevska OP, Golub AA, Prylutskyy YuI, Potebnya GP, Ritter U, Scharff P. Study of C_{60} fullerenes and C_{60}-containing composites cytotoxicity *in vitro*. *Mater Sci Eng C.* 2007; 27(5-8): 1121-1124.
16. Prylutska SV, Grynyuk II, Grebinyk SM, Matyshevska OP, Prylutskyy YuI, Ritter U, Siegmund C, Scharff P. Comparative study of biological action of fullerenes C_{60} and carbon nanotubes in thymus cells. *Mat-wiss u Werkstofftech.* 2009; 40(4): 238-241.
17. Tolkachov M, Sokolova V, Loza K, Korolovych V, Prylutskyy Y, Epple M, Ritter U, Scharff P.

Study of biocompatibility effect of nanocarbon particles on various cell types *in vitro*. *Mat-wiss u Werkstofftech.* 2016; 47(2-3): 216-221.
18. Tsai MC, Chen YH, Chiang LY. Polyhydroxylated C_{60}, fullerenol, a novel free-radical trapper, prevented hydrogen peroxide- and cumene hydroperoxide-elicited changes in rat hippocampus in-vitro. *J Pharm Pharmacol*. 1997; 49(4): 438-445.
19. Youle RJ, Karbowski M. Mitochondrial fission in apoptosis. *Nat Rev Mol Cell Biol*. 2005; 6(8): 657-663.
20. Scharff P, Carta-Abelmann L, Siegmund C, Matyshevska OP, Prylutska SV, Koval TV, Golub AA, Yashchuk VM, Kushnir KM, Prylutskyy YuI. Effect of X-ray and UV irradiation of the C_{60} fullerene aqueous solution on biological samples. *Carbon*. 2004; 42(5-6): 1199-1201.
21. Ritter U, Prylutskyy YuI, Evstigneev MP, Davidenko NA, Cherepanov VV, Senenko AI, Marchenko OA, Naumovets AG. Structural features of highly stable reproducible C_{60} fullerene aqueous colloid solution probed by various techniques. *Fuller Nanotub Carbon Nanostruct*. 2015; 23(6): 530-534.
22. Nozdrenko DM, Bogutska KI, Prylutskyy YI, Korolovych VF, Evstigneev MP, Ritter U, Scharff P. Impact of C_{60} fullerene on the dynamics of force-speed changes in soleus muscle of rat at ischemia-reperfusion injury. *Fiziol Zh*. 2015; 61(2): 48-59.
23. Zhang B, Bian W, Pal A, He Y. Macrophage apoptosis induced by aqueous C_{60} aggregates changing the mitochondrial membrane potential. *Environ Toxicol Pharmacol*. 2015; 39(1): 237-246.
24. Song M, Liu S, Yin J, Wang H. Interaction of human serum album and C_{60} aggregates in solution. *Int J Mol Sci*. 2011; 12(8): 4964-4974.
25. Lyon DY, Adams LK, Falkner JC, Alvarezt PJ. Antibacterial activity of fullerene water suspensions: effects of preparation method and particle size. *Environ Sci Technol*. 2006; 40(14): 4360-4366.
26. Gelderman MP, Simakova O, Clogston JD, Patri AK, Siddiqui SF, Vostal AC, Simak J. Adverse effects of fullerenes on endothelial cells: fullerenol C_{60}(OH)24 induced tissue factor and ICAM-I membrane expression and apoptosis *in vitro*. *Int J Nanomedicine*. 2008; 3(1): 59-68.
27. Prylutskyy Y, Bychko A, Sokolova V, Prylutska S, Evstigneev M, Rybalchenko V, Epple M, Scharff P. Interaction of C_{60} fullerene complexed to doxorubicin with model bilipid membranes and its uptake by HeLa cells. *Mater Sci Eng C Mater Biol Appl*. 2016; 59: 398-403.
28. Didenko G, Prylutska S, Kichmarenko Y, Potebnya G, Prylutskyy Y, Slobodyanik N, Ritter U, Scharff P. Evaluation of the antitumor immune response to C_{60} fullerene. *Mater Wis Werkstofftech.* 2013; 44(2-3): 124-128.
29. Skamrova GB, Laponogov I, Buchelnikov AS, Shckorbatov YG, Prylutska SV, Ritter U, Prylutskyy YI, Evstigneev MP. Interceptor effect of C_{60} fullerene on the in vitro action of aromatic drug molecules. *Eur Biophys J*. 2014; 43(6-7): 265-276.
30. Khoma AM, Zavodovskiy DA, Nozdrenko DM, Dolhopolov OV, Miroshnychenko MS, Motuzjuk AP. Dynamics of ischemic skeletal soleus muscle contraction in rats. *Fiziol Zh*. 2014; 60(1): 34-40. (In Ukrainian).
31. Korthals JK, Maki T, Gieron MA. Nerve and muscle vulnerability to ischemia. *J Neurol Sci*. 1985; 71(2-3): 283-290.
32. Lintz JA, Dalio MB, Joviliano EE, Piccinato CE. Ischemic pre and postconditioning in skeletal muscle injury produced by ischemia and reperfusion in rats. *Acta Cir Bras.* 2013; 28(6): 441-446.
33. Khalilov RA, Dzhafarova AM, Dzhabrailova RN, Emirbekov EZ. Analysis of the kinetic characteristics of lactate dehydrogenase from the rat brain during ischemia and reperfusion. *Neurochem J*. 2014; 8(4): 265-270.
34. Carvalho AJ, McKee NH, Green HJ. Metabolic and contractile responses of fast and slow twitch rat skeletal muscles to ischemia and reperfusion. *Plast Reconstr Surg.* 1997; 99(1): 163-171.
35. Gharbi N, Pressac M, Hadchouel M, Szwarc H, Wilson SR, Moussa F. [60]Fullerene is a powerful antioxidant in vivo with no acute or subacute toxicity. *Nano Lett*. 2005; 5(12): 2578-2585.
36. Markovic Z, Trajkovic V. Biomedical potential of the reactive oxygen species generation and quenching by fullerenes (C60). *Biomaterials.* 2008; 29(26): 3561-3573.
37. Prylutska SV, Skivka LM, Didenko GV, Prylutskyy YI, Evstigneev MP, Potebnya GP, Panchuk RR, Stoika RS, Ritter U, Scharff P. Complex of C_{60} Fullerene with Doxorubicin

as a Promising Agent in Antitumor Therapy. *Nanoscale Res Lett*. 2015; 10(1): 499.

38. Halenova TI, Vareniuk IM, Roslova NM, Dzerzhynsky ME, Savchuk OM, Ostapchenko LI, Prylutskyy YuI, Ritter U, Scharff P. Hepatoprotective effect of orally applied water-soluble pristine C_{60} fullerene against CCl_4-induced acute liver injury in rats. *RSC Adv.* 2016; 6(102): 100046-100055.
39. Prilutski Yu, Durov S, Bulavin L, Pogorelov V, Astashkin Yu, Yashchuk V, Ogul'chansky T, Buzaneva E, Andrievsky G. Study of structure of colloidal particles of fullerenes in water solution. *Mol Cryst Liq Cryst.* 1998; 324(1): 65-70.
40. Prylutskyy YuI, Durov SS, Bulavin LA, Adamenko II, Moroz KO, Geru II, Dihor IN, Scharff P, Eklund PC, Grigorian L. Structure and thermophysical properties of fullerene C_{60} aqueous solutions. *Int J Thermophys.* 2001; 22(3): 943-956.

Received 06.07.2018