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C_{60} fullerene improves the contractile activity of the injured rat *muscle gastrocnemius*

Yuriy Prylutskyy[1], Dmytro Nozdrenko[1], Olexandr Motuziuk[1,2], Svitlana Prylutska[3], Natalia Nurishchenko[1], Daria Franskevych[1], Vasil Soroca[1], Vsevolod Cherepanov[4], Ihor Kalinin[3], Olha Korzhyk[2] and Uwe Ritter[5,*]

[1] ESC 'Institute of Biology and Medicine', Taras Shevchenko National University of Kyiv, 01601 Kyiv, Ukraine
[2] Faculty of Biology and Forestry, Lesya Ukrainka Volyn National University, 43025 Lutsk, Ukraine
[3] Faculty of Plant Protection, Biotechnology and Ecology and Faculty of Veterinary Medicine, National University of Life and Environmental Science of Ukraine, 03041 Kyiv, Ukraine
[4] Institute of Physics, NAS of Ukraine, 03028 Kyiv, Ukraine
[5] Institute of Chemistry and Biotechnology, Technical University of Ilmenau, 98693 Ilmenau, Germany

E-mail: uwe.ritter@tu-ilmenau.de

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Abstract

The powerful antioxidant properties of C_{60} fullerenes have been widely used in biomedical nanotechnology. Owing to the negative effects of free radicals in oxidative stress processes, antioxidants are required to protect injured muscles. Here, the effect of water-soluble C_{60} fullerenes (daily oral dose 1 mg kg^{-1}) on the process of restoration of contractile activity of skeletal muscle of rats (*muscle gastrocnemius*) 15 d after the initiation of open trauma of different severity was studied for the first time. The structural organization of C_{60} fullerene nanoparticles in aqueous solution was analyzed by dynamic light scattering and atomic force microscopy techniques. Such biomechanical parameters of *muscle gastrocnemius* contraction as integrated muscle power, levels of generation of its maximum and minimum force, and time interval until reaching 50% of the level of force response of the muscle were analyzed. Such biochemical indices as concentrations of c-reactive protein, creatinine, and lactate in the rat blood, as well as indices of pro- and antioxidant balance (activities of superoxide dismutase and catalase, the concentration of reduced glutathione) in the blood and muscle tissue of experimental animals, were investigated. It was found that application of water-soluble C_{60} fullerenes statistically significantly improves biomechanical parameters of contraction of injured *muscle gastrocnemius* at the level of 30–45 $\pm$ 3%, which is confirmed by normalization of biochemical indices in the blood and muscle tissue of rats at the level of 35–50 $\pm$ 3% and 20–37 $\pm$ 3%, correspondingly, relative to the open injury group. These findings open the possibility of using C_{60} fullerenes as potential therapeutic nanoagents capable of correcting pathological states of the muscular system during the physiological repair of open injuries.

* Author to whom any correspondence should be addressed.

Keywords: C_{60} fullerene, *muscle gastrocnemius*, open muscle injury, biomechanical parameters of muscle contraction, blood and muscle tissue biochemical indicators, dynamic light scattering, atomic force microscopy

1. Introduction

Open trauma is one of the most common skeletal muscle injuries among both civilians and military personnel [1, 2], and the complications caused by such trauma can be significant [3, 4]. With timely and appropriate treatment ranging from simple closure of injured areas to complex tissue transfer, the threat of limb loss can be prevented [5]. Differences in the severity of the injury sustained and the affected muscle group, as well as the non-specificity of symptoms, complicate research to find effective treatments. In this case, it is important to understand the cellular processes involved in muscle healing. Acute skeletal muscle injury is known to result in fiber destruction, oxidative stress, and inflammation [6, 7]. The complication of healing is often associated with secondary damage to surrounding muscle tissues with the development of inflammatory reactions. The inflammatory reaction depends on two factors, namely the degree of actual physical injury and the degree of muscle vascularization at the time of injury. Studies [8, 9] have shown that the use of various anti-inflammatory treatments is not effective enough to promote the healing of open muscle injuries. Therefore, there is an urgent need to find new techniques and drugs that improve the healing processes of injured muscles [6, 10, 11].

Antioxidant therapy is quite effective in reducing the level of inflammation in muscle injuries [10, 12]. For example, the proanthocyanidin oligomer resveratrol, an antioxidant derived from grape seeds, reduces inflammation in injured muscles [13]. It also improved skeletal muscle regeneration after injury compared to conventional treatment with non-steroidal anti-inflammatory drugs. Melittin, an antioxidant isolated from bee venom, enhances the expression of muscle regeneration factors in a mouse model of skeletal muscle injury [14].

Due to the presence of double electron-deficient chemical bonds in the structure, C_{60} fullerene, as a third allotropic form of carbon, exhibits a strong reducing ability and acts in *in vitro* and *in vivo* systems as a powerful scavenger of free radicals [15–17], the overproduction of which leads to many pathologies. This opens up a real opportunity for the practical use of this nanoantioxidant, with effects surpassing those of well-known natural antioxidants—vitamins C, E, and carotenoids [18] as well as *N*-acetylcysteine and β-alanine [19], which are often used in sports medicine. So, it has been shown that *in vivo* application of an aqueous solution of C_{60} fullerene leads to significant positive therapeutic effects in muscle pathologies of different origins [20–23]. Based on the above data, the present work aimed to investigate the effect of C_{60} fullerenes on the process of recovery of contractile activity of rat skeletal muscle (*muscle gastrocnemius*) 15 d after initiation of open trauma of different severity.

2. Materials and methods

2.1. Preparation of water-soluble C_{60} fullerenes and characterization

A method, based on the transfer of C_{60} molecules from toluene solution to water followed by sonication [24], has been used to preparation of water-soluble C_{60} fullerenes. The resulting fluid at a maximum C_{60} fullerene concentration of 0.15 mg ml^{-1} remains stable for 18 months at a temperature of +4 °C–25 °C.

Measurements of the size distribution for C_{60} fullerenes in aqueous solution and the zeta potential value for them were performed by dynamic light scattering (DLS) on a NanoBrook Omni (Brookhaven, NY, USA) at $T = 298$ K. A DLS instrument equipped with a He–Ne laser (max 5 mW) operating at the wavelength of 512 nm was used. The results were evaluated using the Smoluchowski approximation, which is known to be rigorously valid only for spherical-like particles.

Atomic force microscopy (AFM; 'Solver ProM' system in tapping mode) was used to study the structural organization of C_{60} fullerene in an aqueous solution. To prepare the sample, a drop of water-soluble C_{60} fullerenes was applied to an atomically smooth surface of the mica substrate (SPI Supplies, Grade V1 Muscovite). The visible water evaporation process was monitored using an optical microscope built into the 'Solver ProM' AFM system. The sample was further dried under ambient conditions for 24 h before AFM measurements.

2.2. In vivo *study*

The experiments were conducted on 3-month-old male Wistar rats weighing 170 ± 5 g. All procedures complied with the ARRIVE guidelines. The use of the laboratory animals was approved by the Biomedical Ethics Committee of the ESC 'Institute of Biology and Medicine' of Taras Shevchenko National University of Kyiv (protocol No. 9 dated 4 September 2023) and performed under the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (Strasbourg, 1986) and Article 26 of the Law of Ukraine 'On the Protection of Animals from Cruelty' (No. 3447-IV, 21.02.2006), as well as European Union Directive of 22 September 2010 (2010/63/EU) for the protection of animals used for scientific purposes.

The rats were randomly divided into the following experimental groups: control group ($n = 10$); open injury (non-treated) group 1 ($n = 10$), 2 ($n = 10$), and 3 ($n = 10$) of the 1st, 2nd, and 3rd degree of severity, respectively; open injury + C_{60} (treated) group 1 ($n = 10$), 2 ($n = 10$) and 3 ($n = 10$) of the 1st, 2nd and 3rd degree of severity, respectively (oral daily use of water-soluble C_{60} fullerenes in a

dose of 1 mg kg^{-1} animal body weight after initiation of injury).

Previously we investigated the impact of different doses of C_{60}FAS (0.5, 1, 1.5, and 2 mg kg^{-1}) on various *in vivo* models of muscle pathologies [20–23] and found that the 1 mg kg^{-1} of C_{60}FAS dose demonstrated the high efficacy in the therapy. Therefore, this dose was chosen in the experiments. In addition, the total dose of 15 mg kg^{-1} in the experiment is significantly lower than the LD_{50} value (lethal dose, 50%), which was 600 mg kg^{-1} in the case of oral administration to rats [15] and is therefore safe for bioapplication. It is also important to note that after intravenous administration to mice, the radiolabeled C_{60} fullerenes accumulate predominantly in the blood, spleen, stomach, and liver and are excreted within 72 h, mainly with urine [25, 26].

All experimental studies were conducted on a 16 d post-injury period. This is because the main processes of repairing damaged muscles in the conditions of their natural post-traumatic recovery last for 12–15 d [27].

Anesthesia of rats was carried out by intraperitoneal injection of nembutal (40 mg kg^{-1}), which allowed the animals into deep surgical anesthesia for at least 2 h. *Muscle gastrocnemius* was isolated from surrounding tissues in the area of the hamstring fossa. All branches of the muscle, except for those innervating it, were cut. The isolated muscle was fixed on a bipolar platinum wire electrode for further electrical stimulation. The skin edges on the hind limbs of rats around the incision were sutured to the armature of the strain gauge machine, and the resulting baths with the muscle and nerve were filled with Vaseline oil.

During the experiments, the *muscle gastrocnemius* was transversely dissected by 1, 2, and 3 incisions of 1 mm depth each at three equidistant locations to obtain the 1st, 2nd, and 3rd degree of severity of open muscle injury, respectively [28]. We then sutured the wound skin opening with absorbable synthetic suture material (Liberti, Germany).

2.3. Biomechanical and biochemical studies

Analysis of mechanograms for contractile activity of *muscle gastrocnemius* and biochemical analysis of rat blood were performed 15 d after initiation of open muscle injury.

Stimulation of *muscle gastrocnemius* efferents was carried out by electrical impulses generated using a strain gauge generator. Each series of stimulation consisted of a separate series of rectangular 2 ms pulses with a frequency of 50 Hz and a duration of 6 s. The current strength at which the muscle started to contract was considered as threshold and further stimulation was performed at a strength of 1.3–1.4 threshold. The external load on the muscle was controlled using a mechanostimulator system. The force of muscle contraction was measured using strain gauges [20–23].

In the process of analyzing the obtained results, we used several biomechanical parameters that have a clear tendency to change with increasing severity of muscle pathology [20–23], namely:

- integrated muscle power (S)—numerically calculated area under the power curve using Origin 9.4 software. The analysis of this parameter allows us to evaluate the mechanism of muscle activity formation in the system of equilibrium 'force of contraction—external load', which is a physiological analog of the muscular system performance as a whole;
- the level of generation of the maximum force of muscle contraction (F_{max}). This parameter is an indicator of the general dysfunction of the muscular system. A decrease in the maximum possible force response of the muscle is associated with both disorders in the neural component and myotic components of the pathology under study;
- the level of generation of the minimum force of muscle contraction (F_{min}). This parameter is an indicator of the maximum changes caused by the pathological process in each consecutive contractile act;
- an assessment of muscle fatigue development was performed by calculating the time interval until reaching 50% of the force response level (t_{50}). It should be noted that in the control group of animals, very long (several hours) time frames were required to change this parameter.

The concentrations of c-reactive protein (CRP), creatinine, and lactate, as well as indicators of pro- and antioxidant balance (activity of catalase (CAT) and superoxide dismutase (SOD) and concentration of reduced glutathione (GSH)) in the blood plasma and muscle tissue of rats, as markers of muscle damage, were determined using the clinical diagnostic equipment—biochemical analyzers RNL-200 and JN-1101-TR2 (Netherlands), ABX Micros ESV60 and automatic analyzer Pentra C400 (France).

2.4. Statistics

Statistical evaluation of the experimental results was performed using the procedure of analysis of variances with mixed design. Two between-group factors were supposed: (1) open injury (three levels—the 1st, 2nd, and 3rd degree of severity); (2) water-soluble C_{60} fullerenes treatment (two levels—no and use of C_{60}). The factor of consecutive non-relaxation contractions of the *muscle gastrocnemius* when stimulation is applied was supposed as grouped with ten levels. The Shapiro-Wilk W-test was used to test for normality. Levene's test was used to assess the equality of variances across groups. Multiple pairwise comparisons between different groups and conditions were performed by Bonferroni *post-hoc* test. The differences between the experimental groups were considered significant at $p < 0.05$. Each of the experimental force curves is the result of averaging 10 similar tests. Each biochemical measurement was carried out at least three times. The statistical evaluation was performed by the software package Statistica 8.0 (Dell, USA).

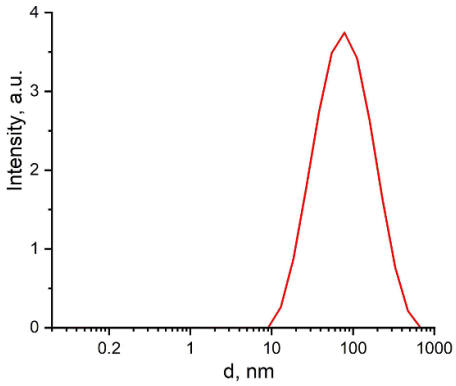

Figure 1. Distribution of the scattered light intensity according to the diameters of light scattering C_{60} fullerene nanparticles (0.15 mg ml^{-1}).

3. Results and discussion

3.1. AFM analysis

Since the size of nanoparticles affects their toxicity and bioactivity [29], we conducted DLS and AFM research on the resulting sample.

A typical result of DLS experiment shown in figure 1 gives the distribution of light-scattering particles according to their hydrodynamic diameters at a fixed solute concentration (0.15 mg ml^{-1}). The main fraction of light-scattering particles (nanoaggregates of C_{60} fullerenes) had diameters in the range of 100 nm.

The magnitude of the zeta potential is related to the stability of colloid dispersions because it determines the degree and nature of the interaction between the particles of the dispersed system. The zeta potential value for the prepared C_{60} fullerene aqueous solution was equal to −24.7 mV. A high negative charge of colloid clusters (or, more strictly, the electrostatic repulsion between the negatively charged clusters) indicates a low degree of particle aggregation over time.

The AFM image obtained (figure 2(A)) shows large C_{60} fullerene nanoclusters with a size of 20–90 nm, which is in good agreement with above DLS results. Moreover, figure 2(B) demonstrates individual C_{60} molecules (diameter ∼0.7 nm) and their small nanoclusters with a size of 1.2–3.0 nm. Thus, the studied aqueous solution is a typical polydisperse nanofluid, which is in good agreement with our previous theoretical calculations [30].

3.2. Biomechanics of injured muscle gastrocnemius contractions

Figure 3 shows the results of changes in the force of 10 consecutive non-relaxation contractions of the *muscle gastrocnemius* of rats 15 d after the initiation of open muscle injury when applying stimulation at a frequency of 50 Hz for a duration of 6 s. The corresponding biomechanical parameters describing the dysfunction of the investigated muscle work were calculated based on the obtained mechanograms (figure 4).

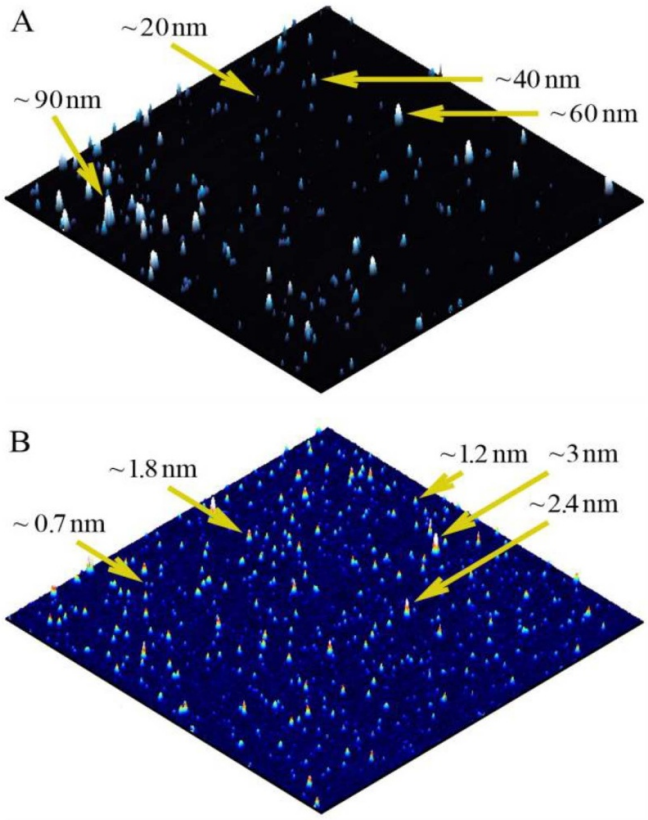

Figure 2. 3D AFM images of C_{60} fullerene nanoparticles in aqueous solution (0.15 mg ml^{-1}).

Change in integrated muscle power is one of the most important parameters of skeletal muscle contraction kinetics [23]. Pathological processes occurring in the nerve or muscle tissue lead to a decrease in this parameter, which indicates the emerging difficulties in accurate joint positioning by the damaged muscle [31].

At the end of 15 d after initiation of open muscle injury of the 1st degree of severity, this parameter was 58 ± 3% and 38 ± 2% (control values were taken as 100%) at the first and tenth contraction, respectively. Application of water-soluble C_{60} fullerenes changed the values of these indices, which were 94 ± 4% and 61 ± 3%, respectively, relative to the control. Significant decrease in integrated muscle power at the 2nd and 3rd degree of injury severity (44 ± 2% and 21 ± 1%, and 40 ± 2% and 15 ± 1%, respectively) was corrected by application of C_{60} fullerenes, namely its increase to 86 ± 4% and 49 ± 3% and 50 ± 3% and 32 ± 2%, respectively, relative to the control (figure 4).

The decrease in integrated muscle power is due to both primary mechanical damage and secondary metabolic damage of myocytes by free radicals. Based on the obtained data, we can assert the positive effect of water-soluble C_{60} fullerenes specifically on secondary, trauma-induced myocyte damage, the recovery of which is usually completed not earlier than after 30 d [32].

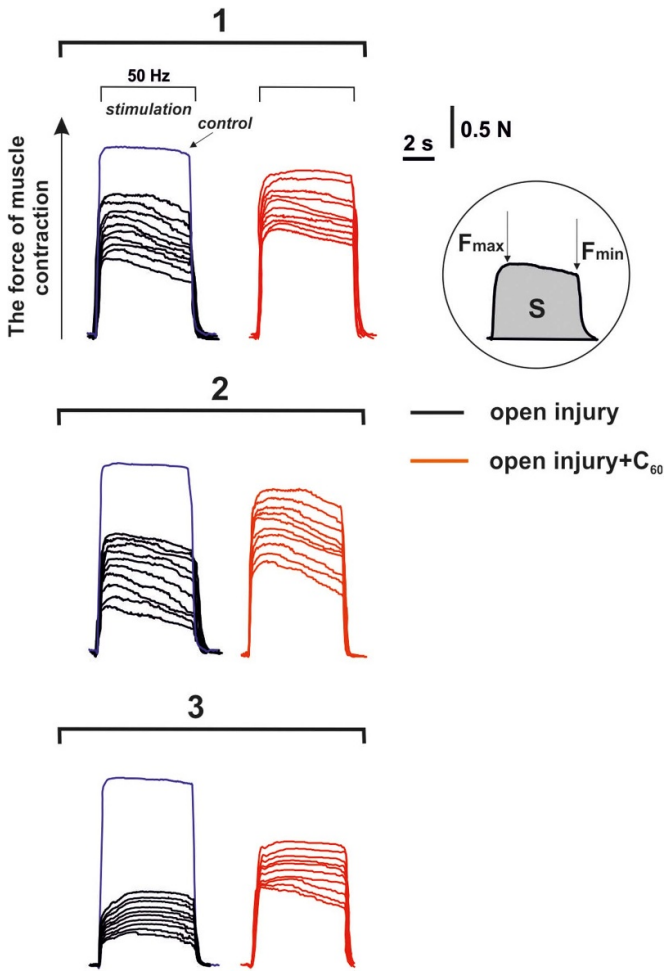

Figure 3. The force of 10 consecutive non-relaxation contractions of rat *muscle gastrocnemius* 15 d after initiation of open muscle injury when applying 50 Hz stimulation of 6 s duration: open injury and open injury + C_{60}—mechanograms of *muscle gastrocnemius* of injured rats and rats receiving water-soluble C_{60} fullerenes after injury initiation; F_{max} and F_{min}—maximum and minimum force of a single muscle contraction, respectively; *S*—integrated muscle power for a single contraction; 1, 2 and 3–1st, 2nd and 3rd degree of severity of open muscle injury, respectively.

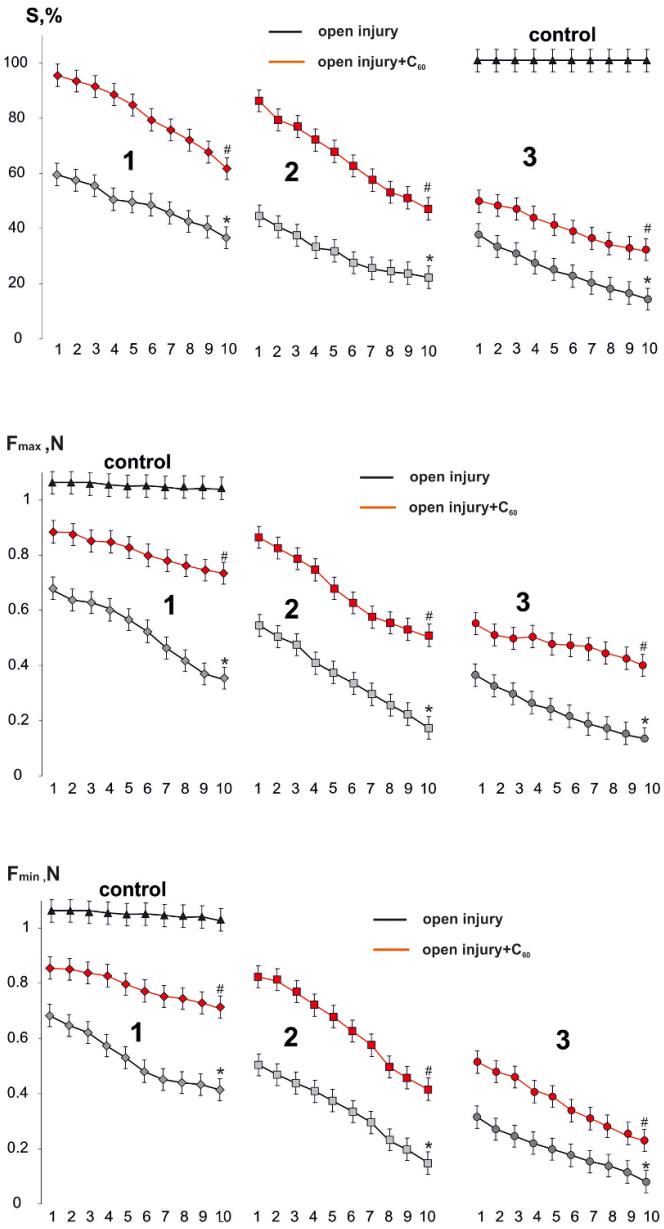

Figure 4. Changes in integrated muscle power, its minimum and maximum force of contraction 15 d after initiation of open injury of rat *muscle gastrocnemius* at the application of 50 Hz stimulation with a duration of 6 s: open injury and open injury + C_{60}—group of injured rats and group of rats receiving water-soluble C_{60} fullerenes after injury initiation, respectively; 1–10—ten consecutive non-relaxation contractions of *muscle gastrocnemius*; 1, 2 and 3–1st, 2nd and 3rd degree of severity of open muscle injury, respectively. $^{*}p < 0.05$ relative to the control group; $^{\#}p < 0.05$ relative to the open injury group.

The level of maximum force of muscle contraction is a biomechanical marker responsible for the adequate realization of motoneuron pools by the muscular system. This component of muscle dynamics is particularly important in the control of hand contraction in humans. Increased intramuscular collagen structures, the presence of non-functioning muscle fibers, inflammatory processes, and the involvement of activated neutrophils releasing additional free radicals into the focus of damage reduce the level of this parameter.

15 d after initiation of open muscle injury, this parameter decreased significantly and was 0.68 ± 0.05 and 0.35 ± 0.03 N at the 1st degree of injury severity, 0.54 ± 0.05 and 0.20 ± 0.02 N at the 2nd degree of injury severity, and 0.40 ± 0.03 and 0.18 ± 0.20 N at the 3rd degree of injury severity on the first and tenth contraction, respectively. In the control, its value was 1.2 ± 0.1 N. Application of water-soluble C_{60} fullerenes increased this parameter to 0.89 ± 0.08 and 0.72 ± 0.07 N, 0.90 ± 0.09 and 0.51 ± 0.05 N and 0.55 ± 0.05 and 0.41 ± 0.04 N at the 1st, 2nd and 3rd degree of injury severity, respectively (figure 4).

Note that the results obtained above can be related not only to the protective effect of antioxidants on myocyte membranes [10, 12, 13]. The trajectory of a rather slow muscle movement

is completely determined by its static properties. If the movement is more rapid, dynamic components are added, which directly depend on the stiffness and pliability of the muscle. It is clear that if complications develop after muscle injuries, they undergo significant changes [23]. Increased fibrosis of skeletal muscles during post-traumatic regeneration worsens their function and negatively affects myocyte regeneration after injury, reducing the maximum possible mechanical force [33]. It can be assumed that the use of C_{60} fullerenes can reduce the level of development of such pathological processes.

The level of minimal muscle contraction force is a biomechanical marker describing the quality level of the performed movement. The inability to maintain the achieved target position throughout the entire muscle activation process leads to serious errors in the positioning system and the occurrence of tremor components [34]. This parameter is not associated with neuropathic damage and its analysis gives an idea of the disturbances of the force generation system within the muscle fiber.

After initiation of open muscle injury, the minimum contraction force decreased from a control value of 1.1 ± 0.1 N to 0.69 ± 0.06 and 0.42 ± 0.04 N at the 1st injury severity level, 0.51 ± 0.05 and 0.21 ± 0.02 N at the 2nd injury severity level, and 0.32 ± 0.03 and 0.18 ± 0.01 N at the 3rd injury severity level on the first and tenth contraction, respectively. Application of water-soluble C_{60} fullerenes increased this parameter, which was 0.85 ± 0.08 and 0.73 ± 0.07 N, 0.81 ± 0.08 and 0.42 ± 0.04 N and 0.53 ± 0.05 and 0.30 ± 0.03 N at the 1st, 2nd and 3rd degree of injury severity, respectively (figure 4).

It should be noted that this parameter is significantly affected by the loss of muscle mass that occurs in the process of posttraumatic regeneration. Significant loss of muscle mass by definition exceeds the endogenous ability to regenerate skeletal muscles, which leads to permanent structural and functional deficits [35], and the positive effect of C_{60} fullerenes on atrophic processes in skeletal muscle was confirmed by us earlier [22].

Muscle fatigue is a protective mechanism against overloading the organism and preventing further development of pathological processes in the muscle [36]. Physiological disorders associated with muscle injury increase their fatigue symptomatology and worsen as the severity of injury increases. To evaluate the effect of C_{60} fullerenes on biomechanical parameters of contraction of injured *muscle gastrocnemius* we analyzed their changes at the onset of muscle fatigue.

Figure 5 presents mechanograms of *muscle gastrocnemius* contraction in rats 15 d after initiation of open muscle injury when applying stimulation at a frequency of 50 Hz for a duration of 2 s in the case of 100 consecutive non-relaxation contractions. On their basis, the indices of the integrated muscle power and reduction time of the maximal force response to 50% of the initial amplitude were analyzed (figure 6).

The integrated muscle power throughout the entire period of stimulation decreased to 45 ± 3%, 40 ± 2%, and 30 ± 2% at the 1st, 2nd, and 3rd degree of injury severity, respectively, relative to the control. It should be noted that at the 3rd degree

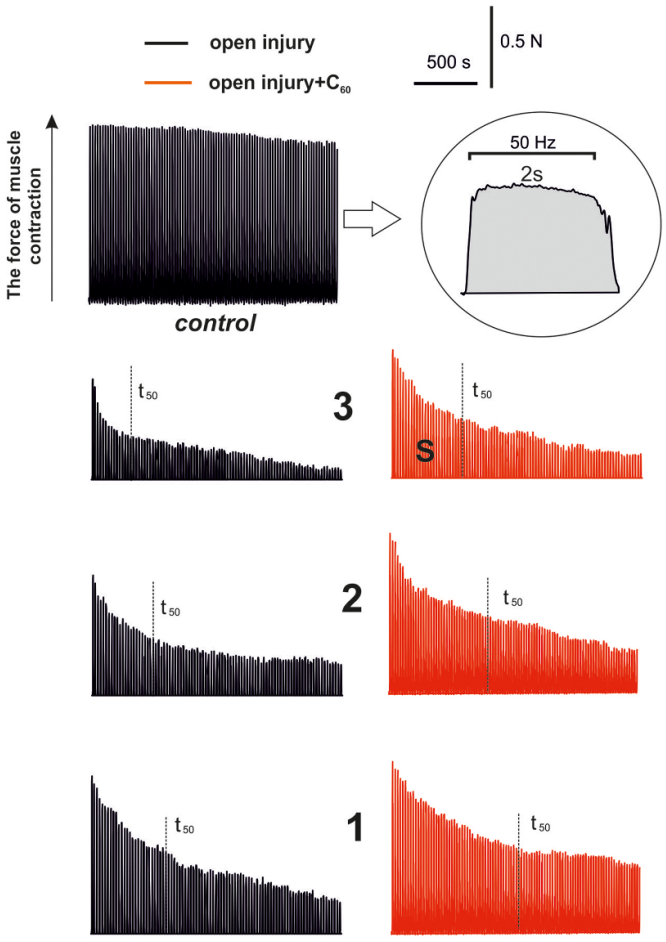

Figure 5. Force generation curves of *muscle gastrocnemius* contractions in rats 15 d after initiation of open muscle injury when applying stimulation at a frequency of 50 Hz for a duration of 2 s in the case of 100 consecutive non-relaxation contractions: open injury and open injury + C_{60}—mechanograms of *muscle gastrocnemius* of injured rats and rats receiving water-soluble C_{60} fullerenes after injury initiation; 1, 2 and 3–1st, 2nd and 3rd degree of severity of open muscle injury, respectively; S—integrated muscle power for the whole contractile process; t_{50}—time to reduce the maximal force response to 50% of the initial muscle force amplitude.

of injury severity the reduction of this parameter after 1200 s ended with almost complete muscle stiffness. Application of water-soluble C_{60} fullerenes increased this parameter, which was 74 ± 4%, 58 ± 3%, and 47 ± 2% at the 1st, 2nd, and 3rd degree of injury severity, respectively, relative to the control. Note that in this case, the muscle responded with a contractile response throughout the entire period of stimulation, not decreasing below the 30 ± 2% limit (figure 5).

The time to decrease the force response of the muscle by 50% of the initial value was 609 ± 11, 501 ± 13, and 408 ± 8 s at the 1st, 2nd, and 3rd degree of injury severity, respectively. Application of water-soluble C_{60} fullerenes significantly increased this parameter, which was 1011 ± 12, 759 ± 15, and 575 ± 9 s at the 1st, 2nd, and 3rd degree of injury severity, respectively (figure 6).

In our opinion, the improvement of these biomechanical parameters of contraction of injured *muscle gastrocnemius* of

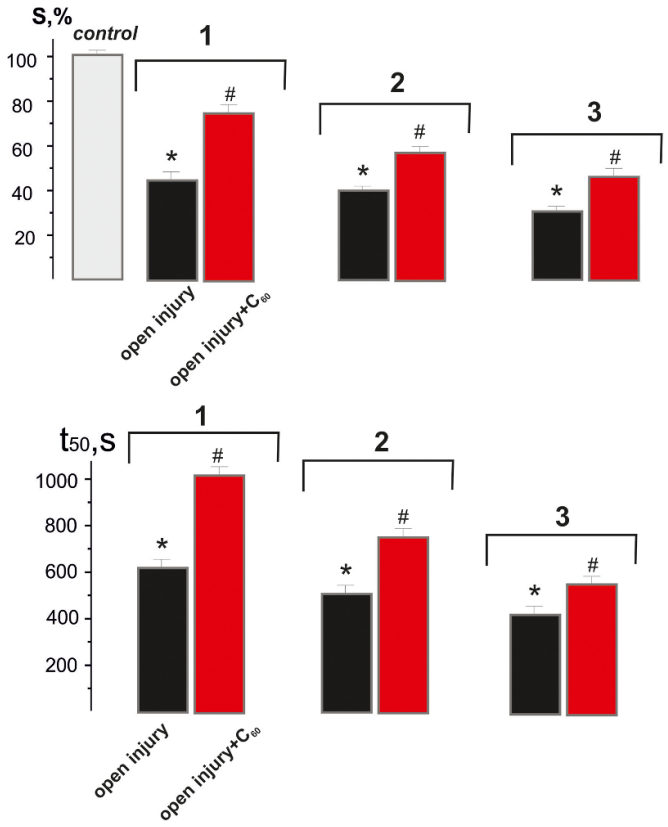

Figure 6. Changes in integrated muscle power and time of maximum force response reduction to 50% of the initial amplitude 15 d after initiation of open injury of rat *muscle gastrocnemius* at the application of stimulation with a frequency of 50 Hz with duration of 2 s in case of 100 consecutive non-relaxation contractions: open injury and open injury + C_{60}—group of injured rats and group of rats receiving water-soluble C_{60} fullerenes after injury initiation, respectively; 1, 2 and 3–1st, 2nd and 3rd degree of severity of open muscle injury, respectively. $^{*}p < 0.05$ relative to the control group; $^{\#}p < 0.05$ relative to the open injury group.

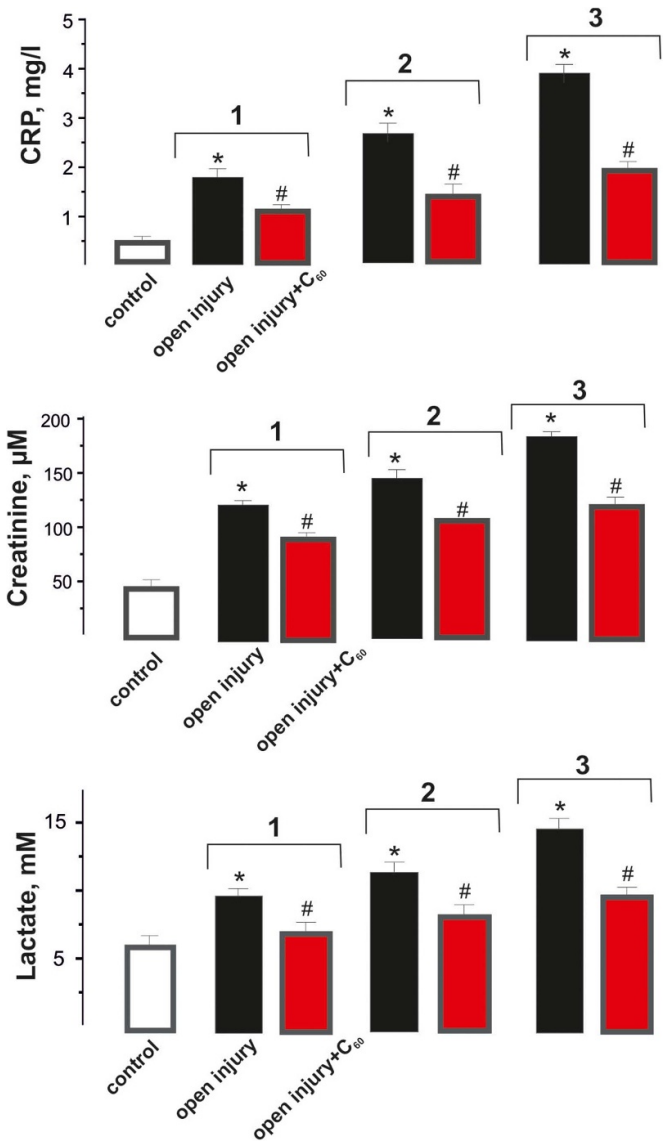

Figure 7. CRP, creatinine, and lactate concentrations in rat blood plasma 15 d after initiation of open *muscle gastrocnemius* injury: open injury and open injury + C_{60}—a group of injured rats and a group of rats receiving water-soluble C_{60} fullerenes after injury initiation, respectively; 1, 2 and 3–1st, 2nd and 3rd degree of severity of open muscle injury, respectively.$^{*}p < 0.05$ relative to the control group; $^{\#}p < 0.05$ relative to the open injury group.

rats upon application of C_{60} fullerenes can be explained precisely by their antioxidant properties [37].

In summary, the application of water-soluble C_{60} fullerenes in a daily dose of 1 mg kg^{-1} during the whole experiment increases the values of investigated mechanokinetic parameters of contraction of injured *muscle gastrocnemius* of rats at the level of 30–45 ± 3% relative to the open injury group.

3.3. Blood and muscle tissue biochemical indicators of rats

To confirm the obtained mechanokinetic data on the contraction of injured *muscle gastrocnemius* at the application of C_{60} fullerenes we analyzed the changes of blood and muscle tissue biochemical composition of experimental animals.

Highly sensitive CRP is a clinical marker of both acute and chronic phases of inflammation in musculoskeletal diseases [38]. It is known that CRP concentration increases in response to the development of muscle injury.

The recorded significant increase in CRP concentration from 0.50 ± 0.03 mg l^{-1} in control to 1.8 ± 0.1, 2.7 ± 0.2, and 3.9 ± 0.3 mg l^{-1} at the 1st, 2nd, and 3rd degree of injury severity, respectively, is evidence of ongoing inflammatory processes in the injured muscle. Application of water-soluble C_{60} fullerenes significantly decreased its concentration to 1.1 ± 0.1, 1.4 ± 0.1, and 2.0 ± 0.2 mg l^{-1} at the 1st, 2nd and 3rd degree of injury severity, respectively (figure 7), which is a confirmation of the prospective application of antioxidants in the therapy of muscle pathologies [17–20].

Creatinine, as a source of energy for muscle contraction, is the end product of creatine and creatine phosphate metabolism. Changes in its concentration are caused by myocyte destruction under various physiological and pathological conditions [39].

Creatinine concentration increased from 43 ± 2 μM in control to 122 ± 6, 149 ± 5, and 181 ± 7 μM at the 1st, 2nd, and 3rd degree of injury severity, respectively. Application of water-soluble C_{60} fullerenes significantly reduced this index to 82 ± 2, 104 ± 3, and 119 ± 3 μM, respectively (figure 7), which, in our opinion, is due to antioxidant properties of C_{60} fullerenes, which protect skeletal muscle cell membranes from nonspecific free-radical destruction by actively absorbing free radicals.

Skeletal muscles are the main producer of lactic acid in the body, but their fibers also use lactic acid as a breathing fuel. The transport of lactic acid across the plasma membrane is fundamental to intracellular pH homeostasis. Lactate is an important metabolic intermediate that can be rapidly exchanged between different cells within a given muscle, between different muscles, and between muscle and blood [40]. An increase in lactate concentration indicates that the amount of lactate entering the cell exceeds its oxidation and excretion. This may be a direct consequence of muscle fiber dysfunction associated with impaired reparative processes after injury.

In control, the lactate concentration was 5.3 ± 0.5 mM. After initiation of open muscle injury, its value increased to 9.2 ± 0.7, 11.9 ± 0.7, and 14.3 ± 0.8 mM at the 1st, 2nd, and 3rd degree of injury severity, respectively. Application of water-soluble C_{60} fullerenes contributed to the increase of lactate oxidation: its concentration significantly decreased and was 7.1 ± 0.3, 7.7 ± 0.3, and 9.8 ± 0.4 mM at the 1st, 2nd and 3rd degree of injury severity, respectively (figure 7).

Muscle damage is usually accompanied by the formation of free oxygen radicals and the intensification of lipid peroxidation (LPO) processes [41]. As a result of biochemical tests, we recorded an increase in the level of markers of peroxidation and oxidative stress (SOD, CAT, and GSH) in the blood and muscle tissue of rats after the induction of injury and their decrease upon application of C_{60} fullerenes (figures 8 and 9).

SOD is one of the most potent endogenous antioxidants, which protects body cells from the damaging effects of free radicals formed during the activation of LPO [42].

SOD activity in blood was 3.7 ± 0.2, 4.3 ± 0.4, and 5.8 ± 0.4 Units ml^{-1} at the 1st, 2nd, and 3rd degree of injury severity, respectively, and 1.8 ± 0.1 Units ml^{-1} in the control. Application of water-soluble C_{60} fullerenes significantly reduced this rate, which was 2.4 ± 0.1, 2.8 ± 0.2, and 3.2 ± 0.3 Units ml^{-1} at the 1st, 2nd, and 3rd degree of injury severity, respectively (figure 8).

SOD activity in muscle tissue was 4.3 ± 0.3, 5.7 ± 0.6, and 8.2 ± 0.5 Units mg^{-1} protein at the 1st, 2nd, and 3rd degree of injury severity, respectively, and 2.6 ± 0.2 Units mg^{-1} protein in the control. Application of water-soluble C_{60} fullerenes significantly reduced this rate, which was 3.1 ± 0.2, 4.0 ± 0.3, and 5.2 ± 0.4 Units mg^{-1} protein at the 1st, 2nd, and 3rd degree of injury severity, respectively (figure 9).

CAT is an enzyme that protects body cells from oxidative damage by reactive oxygen species. The significance of CAT can be judged by assessing its direct or indirect involvement in various diseases [43].

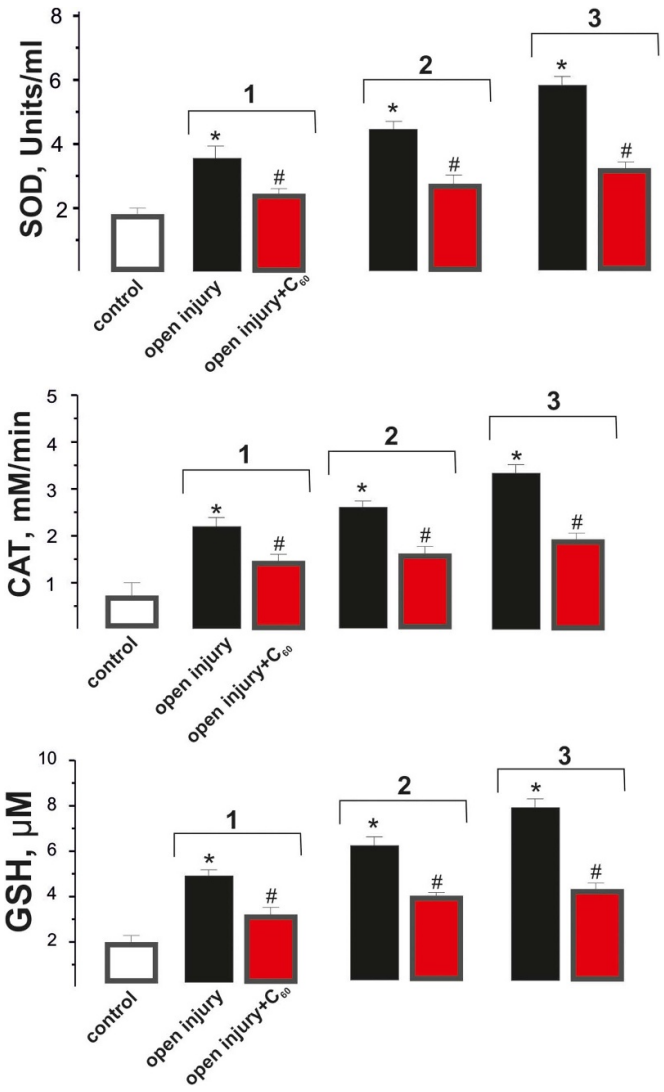

Figure 8. Indices of pro- and antioxidant balance (SOD, CAT, and GSH) in rat blood plasma 15 d after initiation of open injury of *muscle gastrocnemius*: open injury and open injury + C_{60}—a group of injured rats and a group of rats receiving water-soluble C_{60} fullerenes after injury initiation, respectively; 1, 2 and 3—the 1st, 2nd and 3rd degree of severity of open muscle injury, respectively. $^{*}p < 0.05$ relative to the control group; $^{\#}p < 0.05$ relative to the open injury group.

CAT activity in blood after initiation of open muscle injury increased from 0.60 ± 0.05 mM min^{-1} in control to 2.2 ± 0.1, 2.5 ± 0.2, and 3.4 ± 0.3 mM min^{-1} at the 1st, 2nd, and 3rd degree of injury severity, respectively. Application of water-soluble C_{60} fullerenes reduced this index to 1.4 ± 0.1, 1.6 ± 0.1 and 1.9 ± 0.2 mM min^{-1}, respectively (figure 8).

CAT activity in muscle tissue increased from 2.0 ± 0.3 μM/min/mg protein in control to 3.5 ± 0.3, 4.4 ± 0.4, and 5.2 ± 0.5 μM/min/mg protein at the 1st, 2nd, and 3rd degree of injury severity, respectively. Application of water-soluble C_{60} fullerenes reduced this index to 2.8 ± 0.2, 3.2 ± 0.3 and 3.9 ± 0.4 μM/min/mg protein, respectively (figure 9).

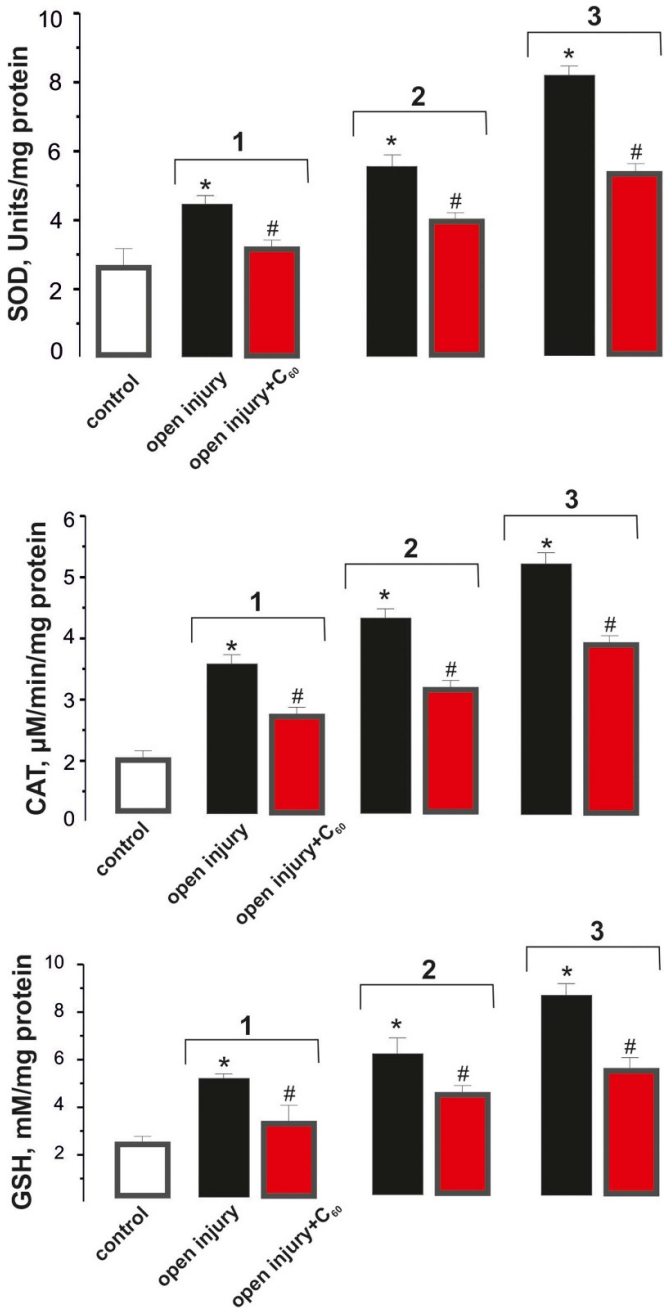

Figure 9. Indices of pro- and antioxidant balance (SOD, CAT, and GSH) in rat *muscle gastrocnemius* tissue 15 d after initiation of open injury: open injury and open injury + C_{60}—a group of injured rats and a group of rats receiving water-soluble C_{60} fullerenes after injury initiation, respectively; 1, 2 and 3—the 1st, 2nd and 3rd degree of severity of open muscle injury, respectively. $^{*}p < 0.05$ relative to the control group; $^{\#}p < 0.05$ relative to the open injury group.

When free radicals are excessively produced, GSH reserves are sharply depleted. The presence of sufficient GSH concentration is a critical factor for cell survival under oxidative stress [44].

After initiation of open muscle injury, GSH concentration in blood was 4.9 ± 0.4, 6.1 ± 0.4, and 7.9 ± 0.5 μM at the 1st, 2nd, and 3rd degree of injury severity, respectively, and 1.9 ± 0.1 μM in the control. Application of water-soluble C_{60} fullerenes reduced this index to 3.1 ± 0.2, 3.9 ± 0.3 and 4.2 ± 0.3 μM, respectively (figure 8).

GSH concentration in muscle tissue was 5.1 ± 0.5, 6.2 ± 0.6, and 8.9 ± 0.8 mM mg^{-1} protein at the 1st, 2nd, and 3rd degree of injury severity, respectively, and 2.2 ± 0.4 μM mg^{-1} protein in the control. Application of water-soluble C_{60} fullerenes reduced this index to 3.2 ± 0.3, 4.7 ± 0.4 and 5.8 ± 0.6 mM mg^{-1} protein, respectively (figure 9).

Thus, the application of water-soluble C_{60} fullerenes in a daily dose of 1 mg kg^{-1} during the whole experiment reduces biochemical indices in the blood and muscle tissue of experimental animals at the level of 35–50 ± 3% and 20–37 ± 3%, correspondingly, relative to the open injury group, thus contributing to the reduction of oxidative processes in the damaged muscles by maintaining the balance between pro-oxidants and antioxidant defense system, preventing the negative effect of free radicals on cellular and subcellular structures during reparative post-traumatic processes in rats.

4. Conclusions

Based on the obtained data, we can conclude that application of water-soluble C_{60} fullerenes (daily oral dose 1 mg kg^{-1}) within 15 d after initiation of open injury of *muscle gastrocnemius* of rats of different severity degree improves its contractile activity, which is confirmed by the increase of values of the studied biomechanical parameters of muscle contractions at the level of 30–45 ± 3% and decrease of biochemical indices in the blood and muscle tissue at the level of 35–50 ± 3% and 20–37 ± 3%, correspondingly, relative to the open injury group. In our opinion, C_{60} fullerenes can influence the activity of endogenous antioxidants, preventing the occurrence of dysfunction in the active muscle and, thus, maintaining it within the physiological norm during the whole process of its contraction. This opens up the possibility of using C_{60} fullerenes as promising nanoparticles—oxidants capable of correcting pathological conditions of the muscular system arising during the repair of open injuries.

Data availability statement

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

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Author contributions

Conceptualization, Y Prylutskyy; Formal analysis, O Motuziuk, and D Nozdrenko; Investigation, D Nozdrenko, O Motuziuk, S Prylutska, N Nurishchenko, D Franskevych, V Soroca, V Cherepanov, I Kalinin, O Korzhyk; Coordination of the research work, Y Prylutskyy and D Nozdrenko; Writing—original draft preparation, D Nozdrenko; Writing—review and editing, Y Prylutskyy and U Ritter; Supervision, U Ritter.

Conflict of interest

The authors have no competing interests or relevant affiliations with any organization or entity with the subject matter or materials discussed in the manuscript.

Writing disclosure

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

All of the *in vivo* experiments were approved by the Bioethics Committee of the ESC 'Institute of Biology and Medicine' of Taras Shevchenko National University of Kyiv (protocol No. 9 dated 4 September 2023) and performed under the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (Strasbourg, 1986) and Article 26 of the Law of Ukraine 'On the Protection of Animals from Cruelty' (No. 3447-IV, 21.02.2006), as well as European Union Directive of 22 September 2010 (2010/63/EU) for the protection of animals used for scientific purposes.

ORCID iDs

Yuriy Prylutskyy https://orcid.org/0000-0002-9847-4137
Dmytro Nozdrenko https://orcid.org/0000-0001-7254-1533
Olexandr Motuziuk https://orcid.org/0000-0002-1520-0721
Svitlana Prylutska https://orcid.org/0000-0001-5280-8341
Natalia Nurishchenko https://orcid.org/0000-0001-8160-8832
Daria Franskevych https://orcid.org/0000-0002-1719-0512
Vasil Soroca https://orcid.org/0000-0002-8340-7636
Vsevolod Cherepanov https://orcid.org/0000-0003-4649-1936
Ihor Kalinin https://orcid.org/0000-0002-3740-7600
Olha Korzhyk https://orcid.org/0000-0002-2526-8877
Uwe Ritter https://orcid.org/0000-0002-9315-4863

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