Heliyon 10 (2024) e32677

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

C_{60} fullerene protective effect against the rat *muscle soleus* trauma

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ARTICLE INFO

Keywords:
C_{60} fullerene
Muscle soleus
Muscle trauma
Biomechanical parameters of muscle contraction
Blood biochemical indicators

ABSTRACT

Muscle trauma is one of the most common body injuries. Severe consequences of muscle trauma are ischemic injuries of the extremities. It is known that the intensification of free radical processes takes place in almost most acute diseases and conditions, including muscle trauma. C_{60} fullerene (C_{60}) with powerful antioxidant properties can be considered a potential nanoagent for developing an effective therapy for skeletal muscle trauma. Here the water-soluble C_{60} was prepared and its structural organization has been studied by the atomic force microscopy and dynamic light scattering techniques. The selective biomechanical parameters of *muscle soleus* contraction and biochemical indicators of blood in rats were evaluated after intramuscular injection of C_{60} 1 h before the muscle trauma initiation. Analysis of the force muscle response after C_{60} injection (1 mg kg^{-1} dose) showed its protective effect against ischemia and mechanical injury at the level of 30 ± 2 % and 17 ± 1 %, accordingly, relative to the pathology group. Analysis of biomechanical parameters that are responsible for correcting precise positioning confirmed the effectiveness of C_{60} at a level of more than 50 ± 3 % relative to the pathology group. Moreover, a decrease in the biochemical indicators of blood by about 33 ± 2 % and 10 ± 1 % in ischemia and mechanical injury, correspondingly, relative to the pathology group occurs. The results obtained demonstrate the ability of C_{60} to correct the functional activity of damaged skeletal muscle.

1. Introduction

Muscle trauma is among the most common injuries to the human body [1]. The problem of determining the severity of myopathic damage continues to be of great concern due to the difficulties in making decisions regarding treatment, determining the quality of the therapy used, as well as the relatively high relapse rate. Therefore, it is necessary to understand the processes at the molecular (cellular) level inherent in a particular type of skeletal muscle damage that are involved in their healing. The most important of these is inflammation. The inflammatory response depends on two factors, namely the actual physical damage and muscle vascularization at the time of injury [2]. All this ultimately affects the immune and cytokine responses of the body and makes it difficult to adequately interpret the mechanisms of the studied pathology.

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https://doi.org/10.1016/j.heliyon.2024.e32677
Received 21 January 2024; Received in revised form 6 June 2024; Accepted 6 June 2024
Available online 7 June 2024

Severe consequences of muscle injuries are ischemic injuries of the extremities. The rapid determination of the degree of ischemia that has occurred is critical for adequate treatment [1,2]. Ischemic tissue damage triggers biochemical reactions that are initiated under hypoxic conditions after a few minutes due to insufficient blood supply and continue for two to 3 h [3]. With ischemia lasting three or more hours, necrotic changes in muscle tissue (the amount of necrosis can reach up to 60 %) as well as nervous degradation occur. Activated neutrophils attracted to a lesion release free radicals, which is a characteristic manifestation of ischemic damage [3,4].

The main clinical method for assessing the viability of muscle fibers is biochemical analysis. Such blood biochemical indicators as levels of creatine phosphokinase (CPK), creatinine, lactate dehydrogenase (LDH) and lactate (LA) are markers of muscle tissue disorders that are used in clinical studies to determine the degree of damage and evaluate therapeutic procedures [5].

The dynamics of muscle contraction is determined by specific mechanisms of interaction between motor neuron pools, entering the muscle, as well as actin and myosin myofilaments. Therefore, an adequate understanding and analysis of changes in neuromuscular pathologies require a comprehensive experimental approach with the possibility of simultaneous control of various biomechanical parameters. Only in this case does it become possible to trace changes in the response of a neuromuscular system to stimulation, which are responsible for the development of precise positional movements, the analysis of which is important in making a conclusion about the efficiency of the therapeutic agent used.

Literature data [6] indicate that free radicals are one of the main factors of muscle tissue damage. They initiate lipid peroxidation (LPO), suppress the ATPase (Adenosinetriphosphatase) activity and mitochondrial respiratory chain enzymes. Hence, antioxidant therapy, including the development of novel effective nanoscale free radical scavengers, remains an attractive strategy for skeletal muscle trauma.

C_{60} fullerene is a fairly strong electronic acceptor capable of absorbing a large number of free radicals [7]. We have previously shown, that water-soluble C_{60} fullerenes prevent the restraint of stress-induced oxidative disorders in rat' brain and heart tissues [8] as well as an acute liver injury [9], diminish muscle fatigue in rats [10], markedly decrease the level of oxidative stress in diet-induced obesity rats [11] and, finally, realize therapeutic effects in the models of muscle injury and ischemia of the *muscle soleus* in rats [12,13]. Recenly the authors [14,15] demonstrated the protective effect of C_{60} fullerene on liver tissue in liver ischemia reperfusion injury as well as on lung and renal tissue in lower extremity ischemia-reperfusion injury in rats, which opens up a prospect for its use in biomedicine.

Taking into consideration the preliminary obtained data, this work aimed to evaluate the protective effect of C_{60} fullerene against the rat *muscle soleus* trauma (mechanical injury and ischemia of varying severity), based on changes in the biomechanical markers of the contractile process and blood biochemical indicators.

2. Materials and methods

2.1. Preparation and characterization of C_{60}FAS

C_{60} fullerene aqueous solution (C_{60}FAS) was prepared by transferring C_{60} molecules from toluene into an aqueous dispersion using an ultrasonic bath [16]. The obtained C_{60}FAS (0.15 mg ml^{-1}) was stable for 18 months at a temperature +4 °C.

The structural organization of C_{60}FAS was investigated by the atomic force microscopy (AFM) and dynamic light scattering (DLS) methods [17]. AFM and DLS measurements were performed on the systems "Solver Pro M" (NT-MDT) and Zetasizer Nano ZS (Malvern Ins. Ltd), correspondingly, at room temperature.

2.2. Animals

Male Wistar rats (170 ± 5 g, 3-month-old) were used in the study. The experimental animals were housed in Plexiglas cages and kept in an air-filtered and temperature-controlled (21 ± 1 °C) room under 12-h light/12-h dark conditions. Rats received a standard pellet diet and water *ad libitum*. 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. 2 dated September 2, 2022) and performed in accordance with 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 animals were randomly divided into the following experimental groups:

- control group (n = 7);
- pathology (non-treated) groups (muscle injury and ischemia with varying degrees of severity (1, 2, and 3), respectively, without drug administration) (n = 7 in each group);
- pathology (C_{60}-treated) groups (intramuscular administration of C_{60}FAS (1 and 2 mg kg^{-1} doses) 1 h before muscle injury and ischemia with different degrees of severity (1, 2 and 3)) (n = 7 in each group).

2.3. Biomechanical and biochemical analyzes

Muscle soleus was released from the surrounding tissues. Its tendon was cut transversely in the distal part, and ventral roots - directly

at their exit points from the spinal cord. Efferents were stimulated by 2 ms (millisecond) electrical pulses generated by a pulse generator. Force of muscle contraction was measured by using strain gauges [12,13].

For muscle ischemization [12], the branch of the femoral artery that provides blood supply to the experimental muscle was tied with ligatures. The duration of ischemization was 1, 2, and 3 h (1, 2, and 3 degrees of ischemia, respectively).

Muscle injury was induced by compressing the muscle for 1, 2, and 3 min (1, 2, and 3 degrees of injury severity, respectively) with a 3.5 mg cm^{-2} pressure clamp [18].

Analyzing the myotic response of the muscle understudy while applying a modulated stimulating signal, we analyzed several basic biomechanical parameters as markers of the muscle dysfunctions [12,13,19], namely:

1. Change in the level of generation of minimum contraction force (F_{min}). This marker is an indicator of the maximum changes in a muscle caused by a pathological process;
2. Change in the time to reach the maximum force response (t_{max}). This marker indicates the level of muscle dysfunction when it performs maximum strength tasks;
3. Change in the level of generation of maximum contraction force (F_{max}). This marker is an indicator of general muscle dysfunction during the development of pathology;
4. Change in the integrated power of muscle contraction (S). This marker, as a calculated area under the force curve, is an indicator of muscle overall performance;
5. Change in the time of reduction of muscle contraction force by 50 % from the initial level (t_{50}). This indicator is used to assess the development of muscle fatigue;
6. Change in the time of force parameters output to the initial level (t_0). This indicator describes a change in the rigid structure of the muscle during the development of pathology;
7. Change in muscle force response onset time (change in latent period; t_{start}). Using this indicator one can evaluate the level of pathology at the stages of the interaction of muscle myofilaments, calcium pump dysfunction, and sarcoplasmic reticulum system.

The levels of enzyme content (CPK and LDH) as well as creatinine, LA, reduced glutathione (GSH), hydrogen peroxide (H_2O_2), thiobarbituric acid reactive substances (TBARS), and catalase (CAT) activity in the blood plasma of experimental animals, as markers of muscle trauma [20], were determined using clinical biochemical equipment (Erba, Czech Republic).

2.4. Statistics

The statistical analysis of the obtained results was performed by using the Statistica 13.3 software. Each of the experimental force curves is the result of averaging 10 similar tests. Each biochemical measurement was carried out at least three times. A factorial ANOVA analysis with Tukey *post-hoc* test was used depending on such factors of variation as dose and degree of muscle trauma severity. Data are presented as the means ± SEM for each group. Differences between groups were considered significant at the level of $p < 0.05$.

3. Results and discussion

3.1. Structural organization of C_{60}FAS

Because specific biological effects are highly dependent on particle size [21,22], we have investigated the size of C_{60} fullerene nanoparticles in a created aqueous solution using the AFM and DLS techniques.

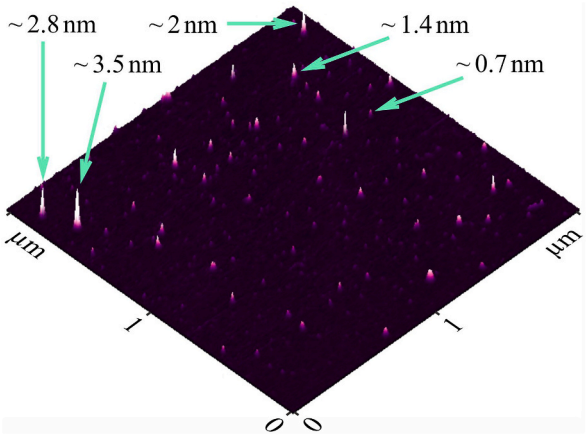

Fig. 1. AFM image of C_{60} fullerene nanoparticles present in C_{60}FAS (0.15 mg ml^{-1}). Values near arrows indicate the height of the nanoobjects.

Fig. 1 demonstrates the 3D AFM image of C_{60} fullerene nanoparticles present in C_{60}FAS, namely a single C_{60} molecule (~0.7 nm (nanometer)) and its nanoclusters (~1.4, ~2.0, ~2.8, and ~3.5 nm).

Fig. 2 shows the DLS data for studied C_{60}FAS. As one can see C_{60}FAS also contains big C_{60} fullerene nanoclusters with following hydrodynamic size ranging: ~64 (8 %), ~75 (32 %), ~82 (42 %) and ~106 (18 %) nm.

3.2. Biomechanics of muscle soleus contraction

The crush syndrome applied as a factor of muscle injury [18] caused a significant decrease in the *muscle soleus* force response (Fig. 3).

Thus, there was a sharp decrease in the *muscle soleus* force response as early in the first seconds of stimulation with a subsequent decrease to 43 ± 3 %, 38 ± 2 %, and 19 ± 2 % in cases of degree 1, 2 and 3 muscle injury, respectively, relative to the control group. The administration of C_{60}FAS (1 mg kg^{-1} dose) increased the muscle force response by 21 ± 2 %, 19 ± 1 %, and 12 ± 1 % for degrees 1, 2, and 3 of muscle injury, respectively, relative to the pathology group. Increasing the C_{60}FAS dose (2 mg kg^{-1}) further marginally increased the strength response by 10 ± 1 %.

After initiation of ischemic injury, the contractile force of the rat *muscle soleus* decreased to 71 ± 4 %, 63 ± 3 %, and 38 ± 3 % in cases of the 1st, 2nd, and 3rd degree of ischemia, respectively, relative to the control group (Fig. 3). C_{60}FAS injections increased the *muscle soleus* force response by 29 ± 2 %, 23 ± 2 %, and 37 ± 2 % at the dose of 1 mg kg^{-1} in cases of the 1, 2 and 3 degree of ischemia, respectively, relative to the pathology group and additionally by 10 ± 1 % at the dose of 2 mg kg^{-1}.

Thus, the applied dose of C_{60}FAS injection of 1 mg kg^{-1} can correct the force response of the muscle with a mechanical injury and ischemia of varying severity. Doubling the dose of the protective agent did not significantly change muscle performance.

The use of biomechanical markers of amplitude-velocity changes in the force response to analyzing the pathological processes understudy has revealed significant qualitative differences between them in the C_{60}FAS applied (Figs. 4 and 5). It turned out that these biomechanical markers of muscle contraction had different sensitivity to the severity of the developing pathology and lined up in the following order: $F_{min} \rightarrow t_{max} \rightarrow F_{max} \rightarrow S \rightarrow t_{50} \rightarrow t_0 \rightarrow t_{start}$.

For example, the change in minimum contraction force (F_{min}) with a muscle injury was 31 ± 2 % and 17 ± 1 % in cases of the 1 and 2 degrees of pathology, respectively, relative to the control group (Fig. 4). The protective effect of C_{60}FAS (dose 1 mg kg^{-1}) on this marker was 60 ± 3 %, 23 ± 1 %, and 18 ± 1 % in cases of the 1, 2, and 3 degrees of pathology, respectively, relative to the pathology group. The change in the time to reach maximum muscle contraction force (t_{max}) with a muscle injury was 42 ± 2 % and 21 ± 1 % in cases of the 1 and 2 degrees of pathology, respectively, relative to the control group (Fig. 3). The protective effect of C_{60}FAS (1 mg kg^{-1} dose) on this marker was 67 ± 3 %, 38 ± 1 %, and 21 ± 1 % in cases of the 1, 2, and 3 degrees of pathology, respectively, relative to the pathology group. The F_{max}, S, and t_{50} markers showed the same tendency to change with C_{60}FAS (1 mg kg^{-1} dose) application in cases of the 1, 2, and 3 degrees of muscle injury (Fig. 3). At the same time, markers t_0 and t_{start} changed only in case of 2 and 3 degrees of the pathological process (76 ± 3 % and 49 ± 2 % of the control group) and recovered by (92–95) ± 3 % when C_{60}FAS (1 mg kg^{-1} dose) was applied (Fig. 3).

When the C_{60}FAS dose (2 mg kg^{-1}) was increased, all of the markers tested responded positively: from 35 ± 2 % for the most sensitive marker F_{min} to 10 ± 1 % for the least sensitive marker t_{start} relative to the pathology group (Fig. 4).

Qualitative changes in muscle dynamics can be traced more significantly when analyzing the protective effect of C_{60}FAS injection on biomechanical markers of muscle contraction during the development of ischemia of varying severity (Fig. 5). So, for example, the change in the minimum contraction force (F_{min}) was 60 ± 3 % and 47 ± 2 % in the case of 1 and 2 degrees of pathology development, respectively, relative to the control. The effect of C_{60}FAS at a 1 mg kg^{-1} dose on this marker was 65 ± 3 %, 56 ± 3 %, and 28 ± 1 % in cases of the 1, 2, and 3 degrees of pathology, respectively, relative to the pathology group. It should also be noted that C_{60}FAS effect is enhanced by (25–30) ± 2 % for the first three sensitive markers (F_{min}, t_{max}, and F_{max}) when the dose is increased to 2 mg kg^{-1}.

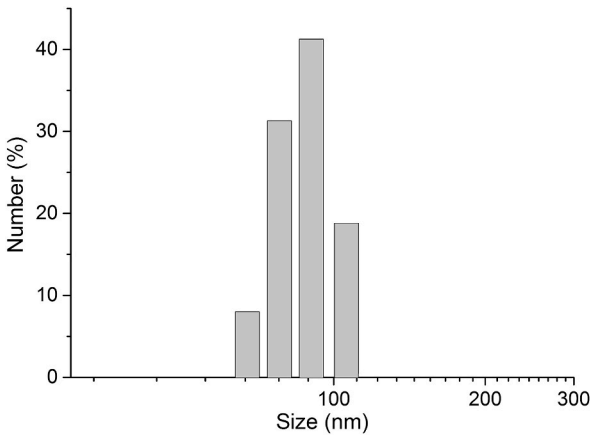

Fig. 2. Distribution of the number of light scattering particles present in C_{60}FAS (0.15 mg ml^{-1}) (in %) according to their hydrodynamic size.

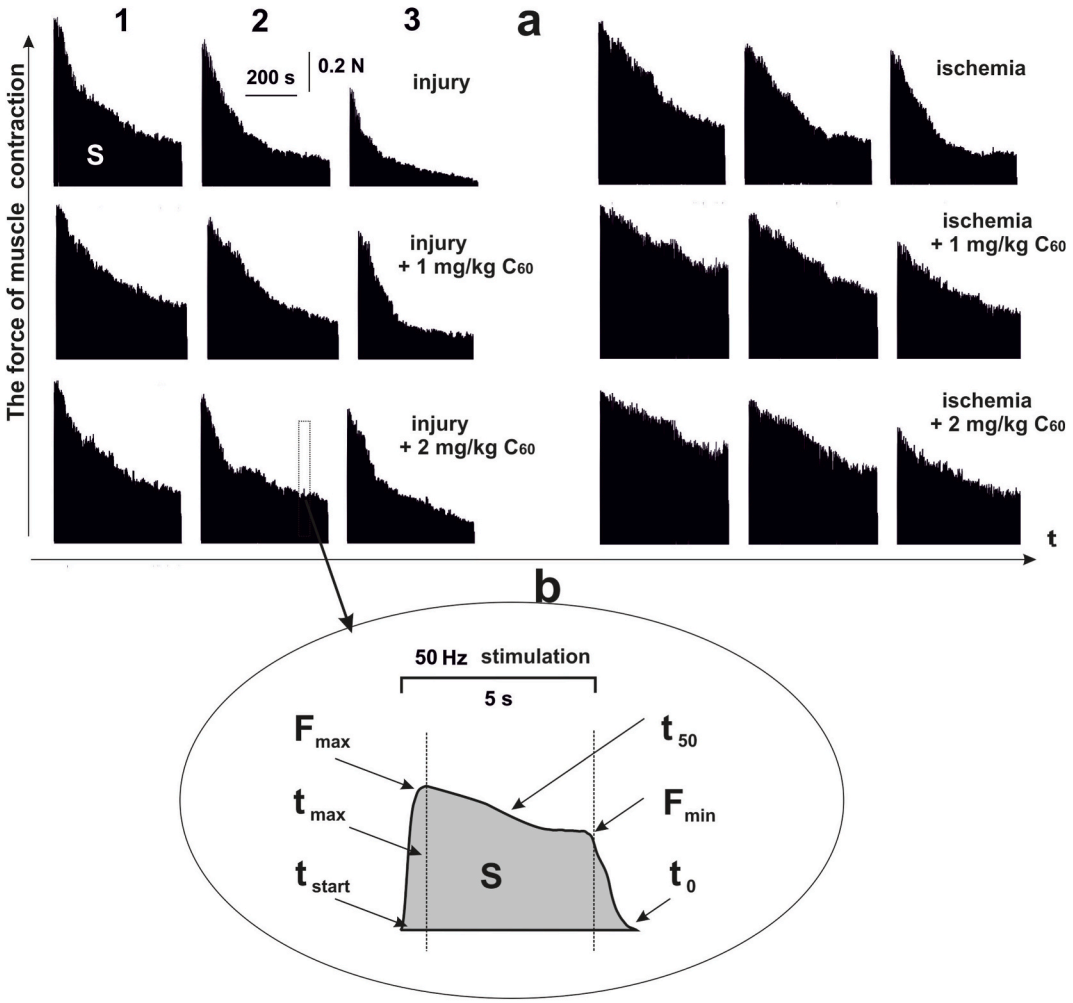

Fig. 3. Force of *muscle soleus* contraction during stimulation at a frequency of 50 Hz for 5 s, duration 500 s without relaxation (a): injury - mechanical injury; ischemia - ischemic muscle; injury/ischemia +1 mg kg^{-1} C_{60} and injury/ischemia +2 mg kg^{-1} C_{60} - corresponding pathology against the background of C_{60}FAS injections at doses 1 and 2 mg kg^{-1}, respectively; 1, 2, 3 – the degree of the pathological process in the muscle. Biomechanical parameters of muscle contraction (*b*): S - integrated muscle power; F_{max} and F_{min} - maximum and minimum forces of a single contraction; t_{max} - time to reach maximum muscle contraction force; t_{50} - time of reduction of muscle contraction force by 50 % from the initial level; t_0 - time of force parameters output to the initial level; t_{start} – time between stimulation start and contractile process start.

Summarizing, one can assume that analysis of selected biomechanical markers in such chronological order of their maximum change makes it possible to determine the severity degree of muscle pathology and the efficiency of the therapy used, which is of paramount importance in emergency conditions.

3.3. Blood plasma biochemical indicators of rats

The analysis of blood biochemical markers, in particular, the levels of creatinine, CPK, LA, and LDH in rat blood plasma (Fig. 6), makes it possible to evaluate physiological changes in the muscle and the therapeutic effect of the drug on pathological processes. As can be seen, they all increase with increasing time after the initiation of injury, which indicates that the muscular system is performing super-intense work for its physiological level, followed by the development of muscle fatigue.

Change in CPK concentration is one of the important markers of pathological processes in skeletal muscles. With mechanical damage to the muscles, there is a release of the CPK from the cells and increase its concentration in the blood. The increase in the blood CPK fraction (Fig. 6a) from 500 ± 15 Units l^{-1} in the control group to 1490 ± 21, 1622 ± 26, and 2430 ± 29 Units l^{-1} in cases of grade 1, 2, and 3 muscle injury, respectively, is the result of myocyte wall destruction with partial release of intramyocyte enzymes into the extracellular space [23]. When C_{60}FAS (1 mg kg^{-1} dose) was injected, CPK levels decreased by 10 ± 1 % in cases of the 1, 2, and 3 degrees of muscle injury, respectively, relative to the pathology group and did not significantly change when the protective dose of C_{60}FAS was increased to 2 mg kg^{-1}.

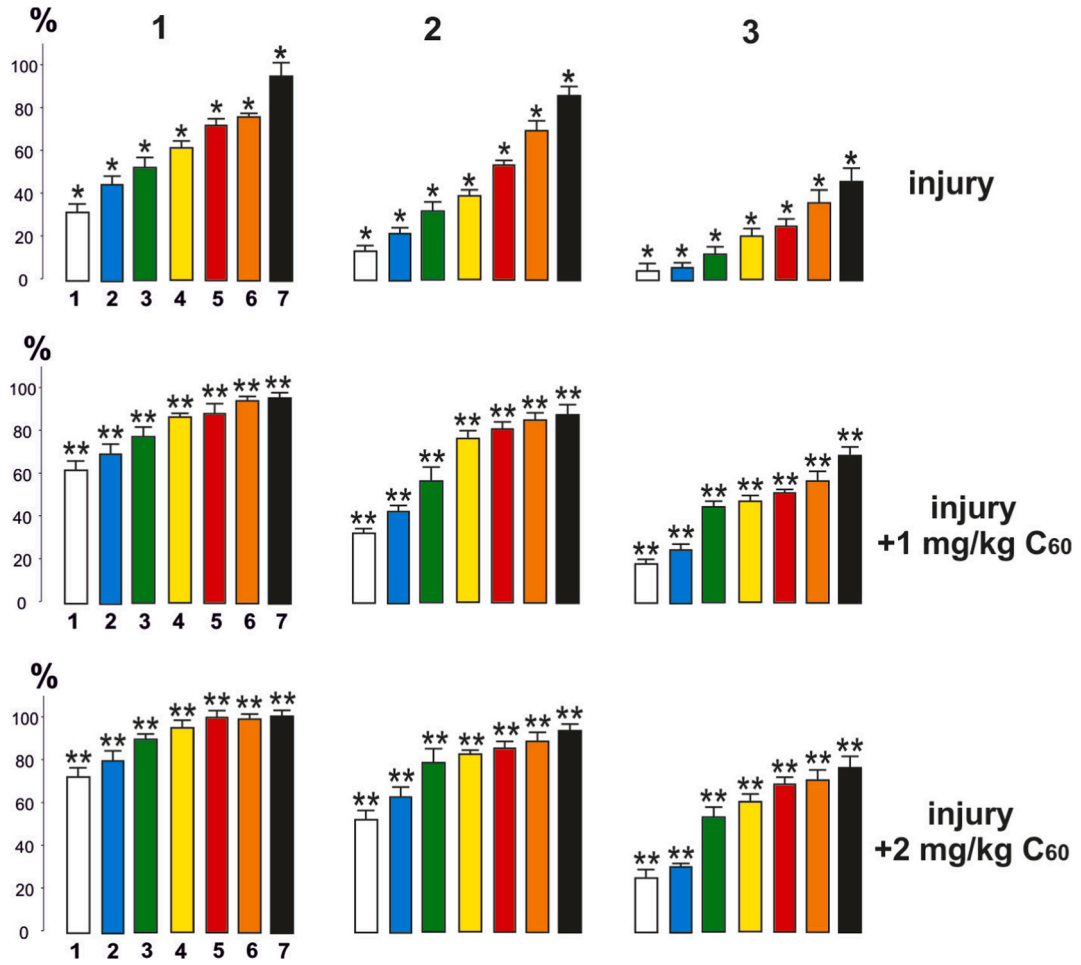

Fig. 4. Biomechanical parameters of muscle contraction (1 - F_{min}; 2 - t_{max}; 3 - F_{max}; 4 - S; 5 - t_{50}; 6 - t_0; 7 - t_{start}) in the order of their maximum change in the analysis of mechanical injury (in % relative to control): injury + 1 mg kg^{-1} C_{60} and injury + 2 mg kg^{-1} C_{60} - mechanical injury against the background of C_{60}FAS injections at doses of 1 and 2 mg kg^{-1}, accordingly; 1, 2, 3 – degree of muscle injury. *$p < 0.05$ vs. control group; **$p < 0.05$ vs. muscle injury group.

During the development of the inflammatory reactions after the initiation of injury, a significant depletion of cellular energy substances (especially ATP) take places, which causes loss of the ion gradient through cell membranes. This leads to the acidification of the pH of intra- and extracellular media [19]. Fig. 6b shows that this parameter increased from 10.3 ± 0.5 M in the control group to 15.7 ± 1, 17.9 ± 1, and 22.4 ± 1 M in cases of the 1, 2, and 3 degrees of muscle injury, respectively. When injected with C_{60}FAS (1 mg kg^{-1} dose), the LA level decreased by 11 ± 1 % in the cases of 1, 2, and 3 degrees of muscle injury, respectively, relative to the pathology group and did not significantly change with an increase in the protective dose of C_{60}FAS up to 2 mg kg^{-1}.

The level of LDH enzyme allows us to assess the general performance status of the damaged muscle after its prolonged activation. The change in the level of LDH from 225 ± 12 Units l^{-1} in the control group to 510 ± 14, 593 ± 9, and 886 ± 15 Units l^{-1}after the initiation of the 1, 2, and 3 degrees of muscle injury indicates the significant muscle dysfunctions associated with excess fatigue pathogens. With C_{60}FAS injection (1 mg kg^{-1} dose), LDH levels decreased by 10 ± 1 % in the cases of 1, 2, and 3 degrees of muscle injury, respectively, relative to the pathology group and did not significantly change when the protective dose of C_{60}FAS was increased to 2 mg kg^{-1} (Fig. 6b).

The relatively low protective effect of C_{60}FAS ((10–14) ± 1 %), revealed by the analysis of changes in blood biochemical indicators, is due to the fact that injury with a muscle tissues rupture is the most severe pathology, covering not only muscle structures but also complicated by high-level pain symptoms. Therefore, the usage of C_{60}FAS, in our opinion, is not a sufficiently complete solution for an adequate therapeutic model. So, the previously discovered synergism of the action of menthol as an analgesic agent and C_{60}FAS on the post-traumatic process of restoring skeletal muscle functions opens up prospects for the clinical application of such combination therapy [13].

Analysis of blood biochemical markers in case of ischemic injuries revealed a significant C_{60} fullerene protective effect. So, when C_{60}FAS (1 mg kg^{-1} dose) was injected, the creatinine level decreased by 31 ± 2 %, 28 ± 2 %, and 21 ± 1 % in the case of 1, 2, and 3 degrees of ischemia, respectively, relative to the pathology group. Increasing the C_{60}FAS dose (2 mg kg^{-1}) additionally improved this

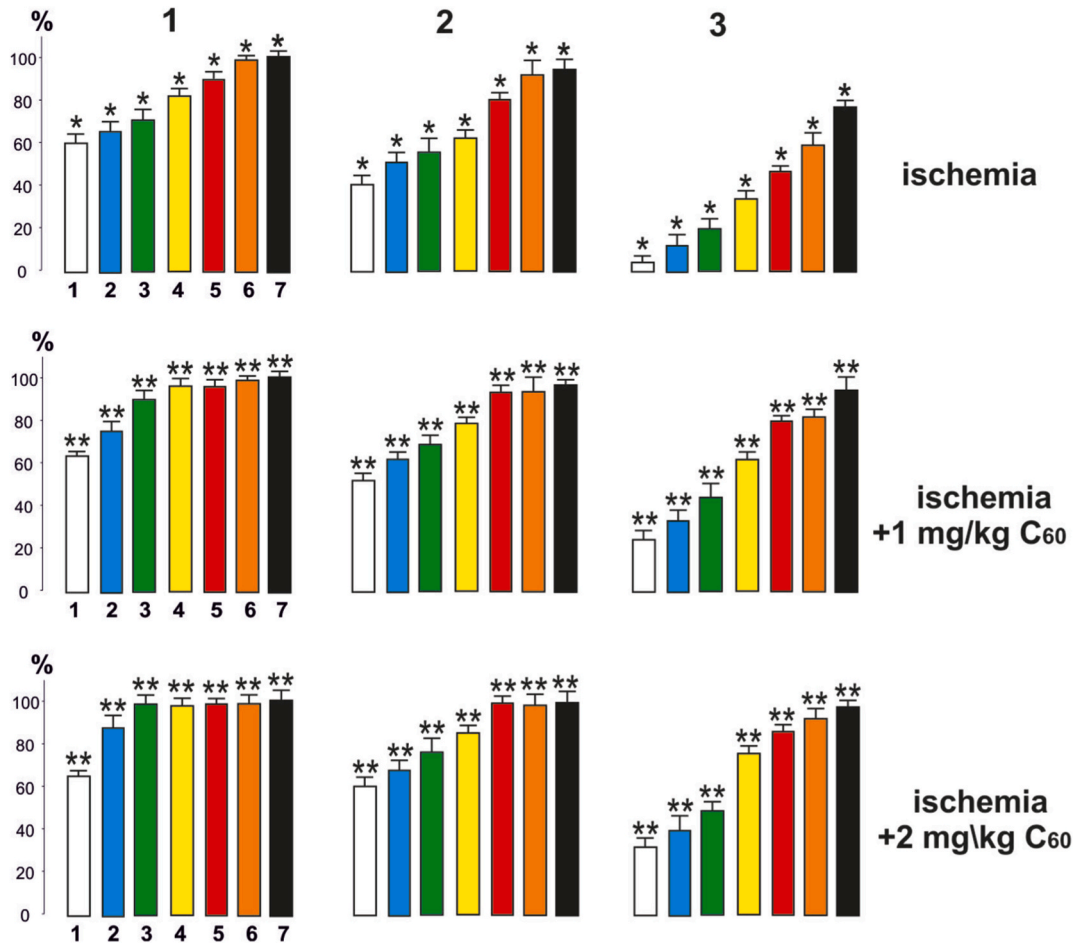

Fig. 5. Biomechanical parameters of muscle contraction (1 - F_{min}; 2 - t_{max}; 3 - F_{max}; 4 - S; 5 - t_{50}; 6 - t_0; 7 - t_{start}) in the order of their maximum change in ischemia analysis (in % relative to control): ischemia +1 mg kg^{-1} C_{60} and ischemia +2 mg kg^{-1} C_{60} - muscle ischemia against the background of C_{60}FAS injection at 1 and 2 mg kg^{-1} doses, accordingly; 1, 2, 3 - degree of muscle ischemia. *$p < 0.05$ vs. control group; **$p < 0.05$ vs. ischemia group.

parameter by an average of 10 ± 1 % (Fig. 6a).

With C_{60}FAS injection (1 mg kg^{-1} dose), the CPK level decreased by 29 ± 1 %, 48 ± 3 %, and 23 ± 1 % in the case of 1, 2, and 3 degrees of ischemia, respectively, relative to the pathology group. Increasing the C_{60}FAS dose (2 mg kg^{-1}) additionally improved this parameter by an average of 10 ± 1 % (Fig. 6a).

When C_{60}FAS (1 mg kg^{-1} dose) was injected, the LA level decreased by 46 ± 3 %, 49 ± 3 %, and 35 ± 2 % in the case of 1, 2, and 3 degrees of ischemia, respectively, relative to the pathology group. Increasing the C_{60}FAS dose (2 mg kg^{-1}) additionally improved this parameter by an average of 13 ± 1 % (Fig. 6b).

With C_{60}FAS injection (1 mg kg^{-1} dose), the LDH level decreased by 47 ± 3 %, 34 ± 2 %, and 26 ± 2 % in the cases of 1, 2, and 3 degrees of ischemia, respectively, relative to the pathology group. Increasing the C_{60}FAS dose (2 mg kg^{-1}) additionally improved this parameter by an average of 10 ± 1 % (Fig. 6b).

Thus, a clear tendency to decreasing the described biochemical blood parameters in the case of ischemic muscle injuries by about (37–56) ± 2 % relative to the pathology group when using C_{60}FAS in a protective regimen takes place. This may indicate activation of the endogenous antioxidant system by C_{60} fullerene nanoparticles, which is confirmed by previously obtained data [9–13,19].

The inflammatory process that occurs immediately after a muscle injury is a source of free radicals and contributes to the LPO intensification [13]. As a result of biochemical tests, we evaluated the indicators of pro- and antioxidant balance (CAT, H_2O_2, TBARS, and GSH) in the blood plasma of rats after the muscle trauma initiation (Fig. 7).

C_{60}FAS injection (1 mg kg^{-1} dose) reduced CAT activity by 10 ± 1 % for muscle injury, and by 33 ± 1 %, 47 ± 2 %, and 22 ± 1 % for ischemia with a grade 1, 2, and 3 pathologies, respectively, relative to the pathology group. Increasing the C_{60}FAS dose (2 mg kg^{-1}) did not significantly change this index for muscle injury and further improved it by 10 ± 1 % for ischemia (Fig. 7a).

The concentrations of H_2O_2 after C_{60}FAS injection (1 mg kg^{-1} dose) decreased by 10 ± 1 % for muscle injury and ischemia - by 29 ± 1 %, 35 ± 1 %, and 22 ± 1 % in the case of 1, 2, and 3 degrees of pathology development, respectively, relative to the pathology

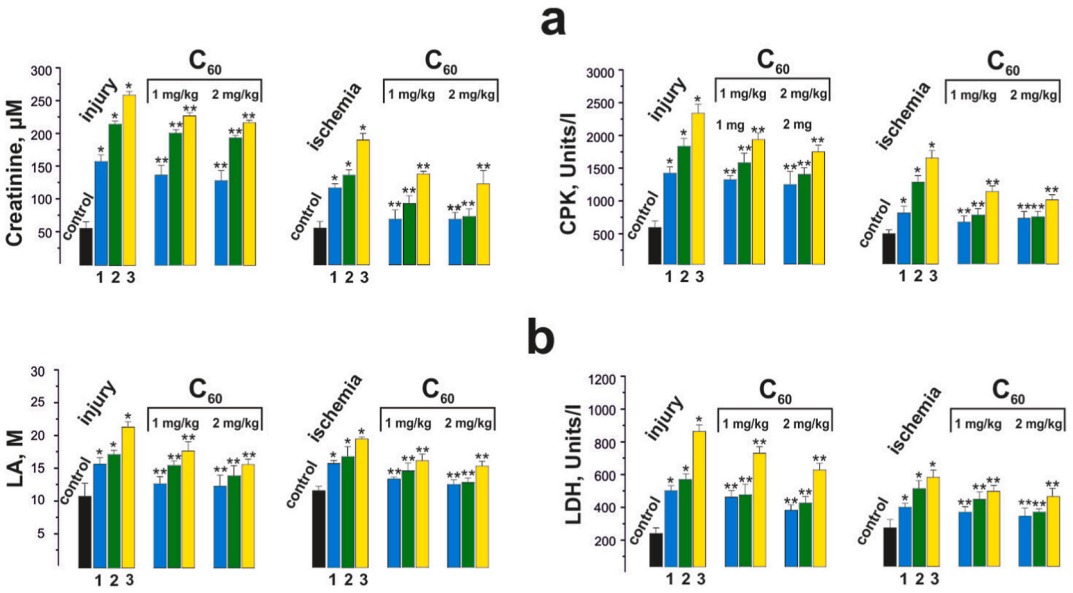

Fig. 6. Biochemical indicators (creatinine and CPK (a), LA and LDH (b) in blood plasma) of the development of pathological processes in the *muscle soleus* of rats: injury - mechanical injury; ischemia - ischemic muscle; C_{60} - the corresponding pathology on the background of C_{60}FAS injection at 1 and 2 mg kg^{-1} doses, accordingly; 1, 2, 3 - the degree of the pathological process development in the muscle; *$p < 0.05$ vs. control group; **$p < 0.05$ vs. the corresponding pathology group.

group. Increasing the C_{60}FAS dose (2 mg kg^{-1}) did not significantly change this parameter for muscle injury and additionally improved it by 11 ± 1 % for ischemia (Fig. 7a).

The concentrations of TBARS after C_{60}FAS injection (1 mg kg^{-1} dose) decreased by 10 ± 1 % for muscle injury and ischemia - by 33 ± 2 %, 38 ± 2 %, and 27 ± 1 % in the case of the 1, 2, and 3 degrees of pathology development, respectively, relative to the pathology group. Increasing the C_{60}FAS dose (2 mg kg^{-1}) did not significantly change this parameter for muscle injury and additionally improved it by 10 ± 1 % for ischemia (Fig. 7b).

GSH concentrations were reduced by 11 ± 1 % for muscle injury and by 29 ± 1 %, 33 ± 2 %, and 25 ± 1 % for ischemia in the case of the 1, 2 and 3 pathology development, respectively, relative to the pathology group when C_{60}FAS (1 mg kg^{-1} dose) was injected. Increasing the C_{60}FAS dose (2 mg kg^{-1}) did not significantly change this parameter for muscle injury and further improved it by 10 ± 1 % for ischemia (Fig. 7b).

Thus, the protective administration of C_{60}FAS reduces the described biochemical parameters for ischemic injuries by (36–43) ± 2 %, and for muscular injuries by (10–13) ± 1 % relative to the pathology group.

Summarizing, the positive effect of C_{60} fullerene injections on the studied muscle pathologies is associated with its powerful antioxidant properties [24]. By inactivation of the free radicals during the development of skeletal muscle trauma, the proposed protective therapy using C_{60} fullerene reduces the degree of its damage.

4. Conclusion

The obtained results indicate that the C_{60}FAS has a pronounced protective effect on the resumption of muscle functional activity after the occurrence of pathological processes associated with muscle trauma. In particular, the analysis of the force muscle response after C_{60}FAS injection (1 mg kg^{-1} dose) showed its protective effect against ischemia and mechanical injury at the level of 30 ± 2 % and 17 ± 1 %, accordingly, relative to the pathology group. Increasing the C_{60}FAS dose (2 mg kg^{-1}) did not significantly increase in the protective effect for both injuries. The analysis of the biomechanical markers responsible for the correction of precise positioning during protective exposure to C_{60}FAS confirmed its effectiveness at the level of more than 50 ± 3 % relative to the pathology group. The use of C_{60}FAS (1 mg kg^{-1} dose) showed a clear tendency to decrease the studied biochemical blood parameters by about 33 ± 2 % and 10 ± 1 % in ischemia and muscle injury, correspondingly, relative to the pathology group. Increasing the C_{60}FAS dose (2 mg kg^{-1}) leads to a reliable increase in the protective effect by another 10 ± 1 % for ischemia and did not significantly change for muscle injury.

Thus, C_{60} fullerenes influence the activity of endogenous antioxidants, preventing the occurrence of dysfunction in the active muscle and, therefore, maintaining it within the physiological norm throughout the contraction process. This opens up the possibility of their usage as potential protective nanoagents capable of correcting the functional muscle activity during its mechanical injury and most efficiently affecting the resumption of the precise control of motor reactions system.

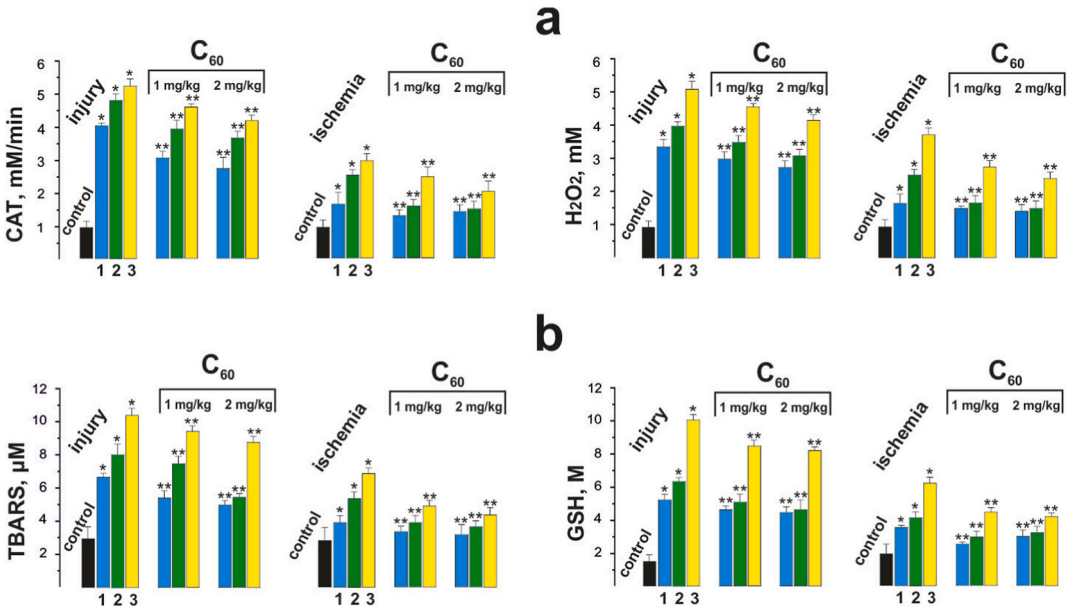

Fig. 7. The prooxidant-antioxidant balance indicators (CAT and H_2O_2 (a), TBARS and GSH (b)) in the blood plasma of rats: injury - mechanical injury; ischemia - ischemic muscle; C_{60} - the corresponding pathology on the background of injections of C_{60}FAS at doses of 1 and 2 mg kg^{-1}, accordingly; 1, 2, 3 - the degree of development of the pathological process in the muscle; $^{*}p < 0.05$ vs. control group; $^{**}p < 0.05$ vs. the corresponding pathology group.

Funding statement

This work was supported by the NRFU (2022.01/0004).

Data availability statement

Data will be made available on request.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Dmytro Nozdrenko: Writing – original draft, Methodology, Investigation, Funding acquisition. **Olexandr Motuziuk:** Investigation. **Svitlana Prylutska:** Methodology, Investigation. **Tetiana Matviienko:** Investigation. **Kateryna Bogutska:** Investigation. **Daria Franskevych:** Investigation. **Nataliya Nurishchenko:** Investigation. **Olga Abramchuk:** Investigation. **Yuriy Prylutskyy:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.

Acknowledgments

The authors are grateful to Prof. U. Ritter (Technical University of Ilmenau, Germany) for kindly providing samples (C_{60}FAS) for research.

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Further reading

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