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ORIGINAL ARTICLE

Post-traumatic recovery of *muscle soleus* in rats is improved via synergistic effect of C_{60} fullerene and TRPM8 agonist menthol

Dmytro Nozdrenko[1] · Tatiana Matvienko[1] · Oksana Vygovska[2] · Vasil Soroca[1] · Kateryna Bogutska[1] · Alexander Zholos[1] · Peter Scharff[3] · Uwe Ritter[3] · Yuriy Prylutskyy[1]

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Abstract

Functional biomechanical parameters of *muscle soleus* contraction in rats as well as selected blood biochemical parameters were studied during the first 3 days of post-traumatic syndrome progression caused by the destruction of muscle cells by compression. Single administration of the antioxidant C_{60} fullerene and the selective agonist of TRPM8 channels menthol were used as therapeutic agents. Injection of C_{60} fullerene at a concentration of 1 mg/kg into the damaged muscle improved its contractile function by 25–28%. The use of combined injections of C_{60} fullerene and menthol (at the concentration 1 mg/kg) improved this index by additional 27–39% and simultaneously stabilized the decrease in muscle strength observed throughout the experiment. A tendency towards a decrease in the indexes of the above described biochemical parameters by 10–15% were found with the therapeutic administration of C_{60} fullerene. With combined injections of C_{60} fullerene and menthol, the above described biochemical parameters decreased by an additional 17–24%. The synergism between the action of menthol and C_{60} fullerene on the post-traumatic recovery of skeletal muscle function opens up new perspectives for the clinical application of this combination therapy.

Keywords Rat *muscle soleus* · Muscle injury · C_{60} fullerene · Menthol · TRPM8 channel · Biomechanical and biochemical parameters

Abbreviations

C_{60}	C_{60} fullerenes
C_{60}FAS	C_{60} fullerene aqueous solution
CAT	Catalase
CS	Crush syndrome
CPK	Creatine phosphokinase
H_2O_2	Hydrogen peroxide
GSH	Reduced glutathione
LA	Lactate
LDH	Lactate dehydrogenase
ROS	Reactive oxygen species
TBARS	Thiobarbituric acid reactive substances
TRP channels	Transient Receptor Potential channels

✉ Yuriy Prylutskyy
prylut@ukr.net

Dmytro Nozdrenko
ddd@univ.kiev.com

Tatiana Matvienko
tamatvienko@gmail.com

Oksana Vygovska
ovvigovskaya@gmail.com

Vasil Soroca
vmsoroka@gmail.com

Kateryna Bogutska
biophys@univ.kiev.ua

Alexander Zholos
a.zholos@univ.net.ua

Peter Scharff
peter.scharff@tu-ilmenau.de

Uwe Ritter
uwe.ritter@tu-ilmenau.de

[1] ESC "Institute of Biology and Medicine", Taras Shevchenko National University of Kyiv, Kyiv 01601, Ukraine

[2] Bogomolets National Medical University of Kyiv, Shevchenko Av. 13, Kyiv 01601, Ukraine

[3] Institute of Chemistry and Biotechnology, Technical University of Ilmenau, Weimarer str. 25, 98693 Ilmenau, Germany

Introduction

Despite the high medical and social significance of skeletal muscle injuries, there are quite a few clinical options for their treatment. Timely therapy, especially at the early stages of such pathology, can optimize the regeneration and healing of the damaged skeletal muscles and help prevent the risk of some serious post-traumatic complications while accelerating full muscle recovery. The reaction of the skeletal muscle tissue to the action of the harmful factor(s) has a distinct phase character and is manifested by alteration (stage of inflammation), exudation (release of fluid and blood cells from blood vessels into tissues and organs) and proliferation (recovery phase). In parallel with muscle tissue disorders there is a complex of vascular changes in the form of short-term spasm, arterial and venous hyperaemia. The occurrence of an inflammatory reaction is a consequence of the appearance of a large number of free radical agents in damaged tissues, which trigger a cascade of pathological processes resulting primarily in altered integrity of cell membranes. It is well established that free radicals, particularly superoxide and hydroxyl radicals, are the main factors in the process of muscle tissue damage. Thus, they initiate lipid peroxidation, cause direct inhibition of mitochondrial enzymes of the respiratory chain and ATPase activity, inactivation of glyceraldehyde-3-phosphate dehydrogenase and membrane sodium channels (Cuzzocrea et al. 2001). Biocompatible and bioavailable carbon nanoparticles C_{60} fullerenes (C_{60}) (Halenova et al. 2020; Prylutska et al. 2007) can act as powerful scavengers of free radicals (Eswaran et al. 2018; Gonchar et al. 2018) induced by ischemia–reperfusion injury (Amani et al. 2017; Matvienko et al. 2017). However, our earlier studies (Nozdrenko et al. 2017) were related to relatively mild muscle pathologies. Muscle injury with rupture of muscle tissue is a severe pathology complicated by pronounced pain symptoms. Therefore, the use of C_{60} therapy, in our opinion, is not a sufficiently comprehensive approach for an adequate model of the forthcoming study.

The ability to perceive temperature stimuli provides a basis for the formation of adaptive responses aimed at the active elimination of the pathological process. In recent decades, several members of the superfamily of Transient Receptor Potential (TRP) channels have been identified as specific thermoreceptors. Accumulating evidence indicates their potential participation in a number of physiological processes that contribute to the alleviation of pathological conditions (Nilius et al. 2007; Zholos et al. 2011). Thus, it has been established that menthol increases muscle endurance during exercise, reducing levels of lactic acid and triglycerides in the blood by activating TRPM8 channels, and thus improving energy metabolism of skeletal muscles (Chen 2018 et al.). Long-term studies have shown that therapeutic use of a menthol-containing drug significantly reduces time for return to sports activity in athletes with injuries of varying severity (Isbary et al. 1983). Positive effect of menthol on the rate of the strength recovery of muscles contraction of the athlete's lower body after physical exertion was established (Gillis et al. 2020). In the context of muscle trauma, it is especially relevant that TRPM8 agonist can normalize blood circulation by exerting dual effect on vascular tone – vasorelaxation of constricted blood vessels and vasoconstriction of blood vessels at rest (Johnson et al. 2009).

Based on these observations, the aim of this work was to investigate the possibility of a synergistic effect of the therapeutic action of C_{60} as an antioxidant and menthol as an activator of TRPM8 channels, on post-traumatic restoration of the functioning of the *muscle soleus* in rats.

Materials and methods

To obtain the C_{60} fullerene aqueous solution (C_{60}FAS) we used a method based on the transfer of these carbon nanostructures from toluene to water, followed by sonication (Ritter et al. 2015; Scharff et al. 2004). The obtained C_{60}FAS is a typical colloid solution that contains both single C_{60} (~0.72 nm) and their nanoaggregates with a size of 1.2–100 nm (Prilutski et al. 1998). C_{60}FAS was stable at 4 ^{0}C for 18 months.

50 male Wistar rats aged 3 months weighing 170 ± 5 g were used in the experiments. The study protocol was approved by the Bioethics Commission of ESC "Institute of Biology and Medicine", Taras Shevchenko National University of Kyiv in accordance with the European Convention for the Protection of Vertebrates animals used for experimental and other scientific purposes" and norms of biomedical ethics in accordance with the Law of Ukraine №3446—IV 21.02.2006, Kyiv, "On protection of animals from cruel treatment" during medical and biological research.

The animals were anaesthetized by intraperitoneal administration of nembutal (40 mg/kg). Muscle injury was induced by squeezing the muscle for 1 min with a clamp at a pressure of 3.5 kg per cm^3 (Souza et al. 2013). The applied crush syndrome (CS) led to the systemic manifestation of pathological changes due to destruction of muscle cells, particularly, the release of muscle cell components (creatine kinase, lactic acid, myoglobin) into the extracellular environment, which served as a marker of muscle injury.

Preparation of the experiment included cannulation (*a. carotis communis sinistra*) for the pharmacological drugs administration and measurement of blood pressure, tracheotomy and laminectomy at the level of the lumbar spinal cord.

The rat *muscle soleus* was separated from the surrounding tissues. In the distal part, its tendon part was cut transversely. For modulated stimulation of efferents in segments L7-S1, the ventral roots were cut at the points of their exit from the spinal cord. Stimulation of efferents was performed by electrical pulses lasting 2 ms, generated by a pulse generator through platinum electrodes. Control of the external load on the muscle was performed using a system of mechanical stimulators. Perturbation of the load was carried out by a linear electromagnetic motor (Nozdrenko et al. 2018).

To induce muscle contraction, a three-component stimulation signal with a frequency of 1 Hz was used, each lasting 10 min with a relaxation period between pools of 100 s. The strength of the current at which the muscle began to contract was considered as threshold, further stimulation was performed at strength of 1.3–1.4 threshold.

C_{60}FAS and menthol were administered sequentially intramuscularly at a concentration of 1 mg/kg body weight immediately after initiation of muscle injury. It is important to note, that accordingly our previous study, the maximum tolerated dose of C_{60}FAS is 721 mg/kg for *i.p.* administration to mice (Prylutska et al. 2019).

To record the force of skeletal muscle contraction, we used a custom made strain gauge, which included force and length sensors, a synchronous pulse generator and a thermal control system (Nozdrenko et al. 2017).

Integrated muscle power, which is an indicator of the general performance of the muscle during the application of stimulation pools, was calculated as the area under the force curve. The analysis of this parameter made it possible to assess the mechanisms of the formation of muscular activity at the equilibrium state in the "force—external load" system, which is a physiological analogue of the performance of the muscular system as a whole. Mechanograms were analyzed on days 1, 2, and 3 after muscle injury.

The development of muscle contractile activity was assessed by the method of calculating time intervals during which 50% and 25% of the initial levels of force responses were reached during muscle stimulation.

The level of biochemical changes in the blood of experimental animals (creatinine, creatine phosphokinase (CPK), lactate (LA), lactate dehydrogenase (LDH), thiobarbituric acid reactive substances (TBARS), hydrogen peroxide (H_2O_2), reduced glutathione (GSH) and catalase (CAT)), as markers of muscle injury, was determined using clinical diagnostic equipment—a haemoanalyzer (Nozdrenko et al. 2017).

Statistical analysis

Statistical assessment of the results was performed by methods of variation statistics using OriginPro 2020 (v. 9.7) (OriginLab, Northampton, MA, USA). At least six replicates were performed for each measurement. Data are expressed as the means ± SEM for each group. The differences among experimental groups were detected by one-way ANOVA followed by Bonferroni's multiple comparison test. Differences at $p < 0.05$ were considered significant.

Results and discussion

Biomechanics of injured muscle contractions

Applied crush syndrome (CS), as a factor of muscle injury, caused a significant decrease in the force response of the muscle with progressive temporal symptoms (Fig. 1). Thus, force responses to a stimulus showed sharp decrease in force activity of the muscle already in the first seconds of stimulation with progressive decrease in the maximum force to 21 ± 1% of initial values on the first pool of stimulation, 17 ± 1% and 9 ± 1% during the second and third pools, respectively ($n = 10$). The decrease in integrated muscle contraction power was 53 ± 2%, 42 ± 1%, and 23 ± 1% ($n = 10$) in the first, second, and third stimulation pools, respectively (Fig. 1). The time to reach 50% and 25% of the initial level of force response was 156 ± 5 and 401 ± 2 s during the first stimulation pool, 143 ± 3 and 376 ± 3 s during the second and 122 ± 2 and 311 ± 2 s ($n = 10$) during the third stimulation pool. Thus, there are progressive fatigue processes of the injured muscle and insufficient relaxation time for its adequate functioning. It is important to note that the intact muscle under these conditions does not change its strength characteristics during stimulation for several hours (Nozdrenko et al. 2017).

The use of C_{60} injections increased the muscle strength response on average by 9–12% during the first stimulation pool. During the second and third stimulation pools, the muscle strength response did not significantly increase and averaged 5–7% of the control values (Fig. 1, injury + C_{60}). Menthol (M) injections alone did not result in any significant changes in muscle dynamics (Fig. 1, injury + M)).

The use of combined injections of C_{60} and menthol showed a significant increase in muscle strength during all three stimulation pools. The integrated power increased by 58 ± 1%, 42 ± 2% and 36 ± 2% during the first, second and third pool, respectively ($n = 10$). The time to reach 50% and 25% of the initial level of force response increased by almost 50% in each of the three stimulation pools (Fig. 1, injury + C_{60} + M).). It is also important to note the absence of a sharp decrease in the maximum force of contraction during the first seconds of stimulation: the decrease in force occurred smoothly and monotonously during all three stimulation pools.

Next we analyzed the changes in muscle dynamics on days 1, 2, and 3 after muscle injury. The decrease in the

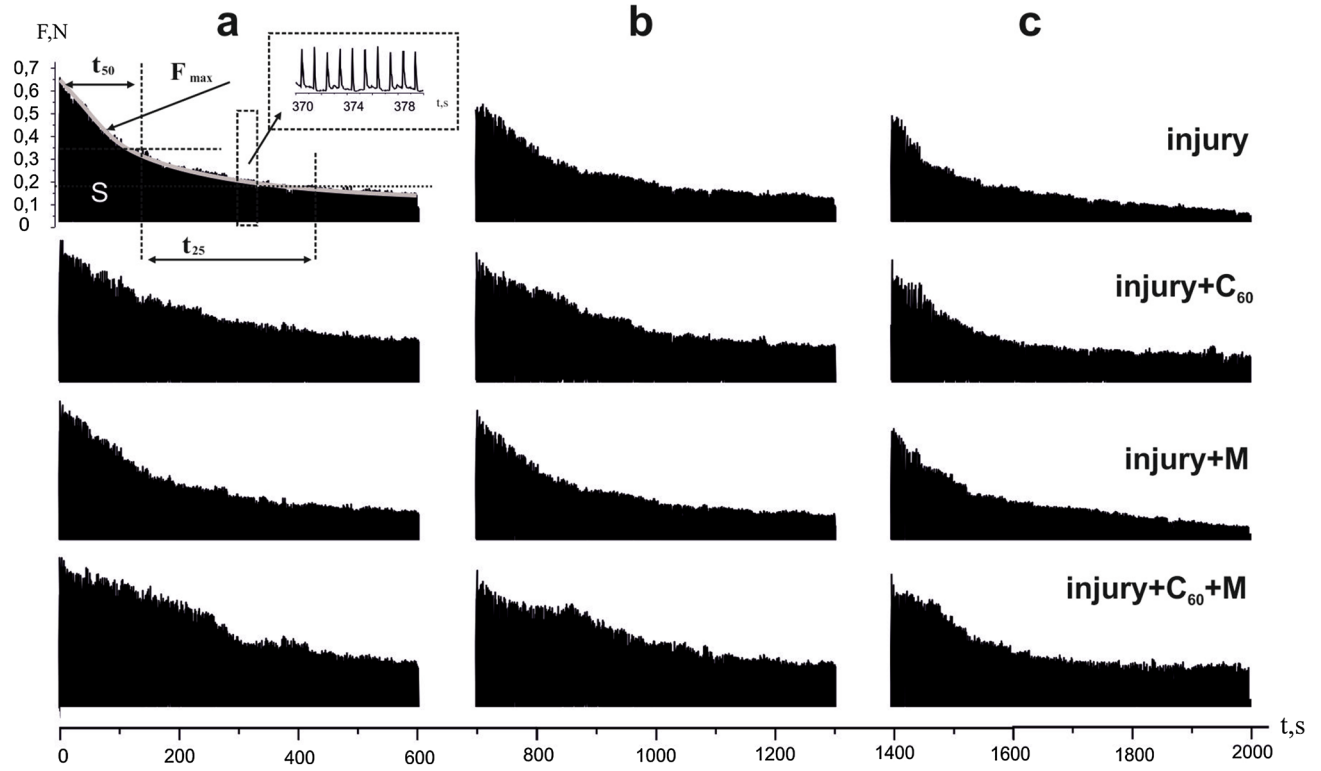

Fig. 1 Curves of the generation of the contraction force of the *muscle soleus* 5 h after the initiation of traumatic injury caused by a three-component stimulation pulses applied at 1 Hz, 600 s duration each with a relaxation period between pools of 100 s: a, b, c—three consecutive pools of stimulation; S is the integrated power of muscle contraction; F_{max}—curve of the maximum force response of the muscle (N); t_{50} and t_{25}—time of the decrease in the maximum strength response to 50% and 25% of the initial amplitude of force of muscle contraction, respectively; "injury + C_{60}" and "injury + C_{60} + M" indicate treatments by injections of C_{60} and C_{60} with menthol (M), respectively

integrated power of muscle contraction was by 46 ± 1%, 31 ± 1% and 15 ± 1% ($n = 10$) of the control values on days 1, 2, and 3 after muscle injury, respectively. The time of the reduction in the maximum power indicators by 50% was 100 ± 2, 78 ± 1 and 54 ± 2 s ($n = 10$), and by 25%—121 ± 1, 107 ± 1 and 78 ± 2 s ($n = 10$) by 1, 2 and 3 days after the injury, respectively (Fig. 2). A temporal analysis of the healing of *muscle soleus* injury showed that complete muscle regeneration occured 3–5 days after the injury (Hurme et al. 1991).

The use of C_{60} injection significantly improved the dynamics of the contractile process of the damaged muscle. Thus, the decrease in the integrated power was by 63 ± 2%, 58 ± 2% and 42 ± 2% ($n = 10$) of the maximum values of the force on days 1, 2, and 3 after the injury, which was 27–30% less than for the injured muscles without treatment. The time to reach the maximum power indicators of 50% of the level from the initial values was 256 ± 11, 321 ± 9 and 211 ± 5 s ($n = 10$), and 25% of the initial level—325 ± 9, 301 ± 7 and 276 ± 6 s ($n = 10$) on days 1, 2 and 3 after the injury, respectively, which was by 30–32% more compared to the injured muscle in control (Fig. 2). Thus, these data indicate a significant positive dynamics of the therapeutic administration of C_{60}FAS. However, it should be noted that this therapy alone did not lead to significant biomechanical changes regarding the nature of the contractile processes. We suppose that inactivation of the produced free radicals by C_{60} reduces the level of injury severity in the muscle by 25–30%, which, although it is a positive aspect of this therapy, does not ensure a significant progress in the process of complete recovery of muscle function.

Menthol injections into the injured muscle did not show much positive therapeutic results in improving its dynamic response (data not shown). However, combined treatment with menthol and C_{60} resulted in the decrease of the integrated power by 79 ± 2%, 63 ± 2% and 49 ± 2% ($n = 10$) of the maximum response on days 1, 2 and 3 after the injury, which was by 40–45% less than for the damaged muscle in control and by 17% less compared to the C_{60} therapy alone. The time to reach the maximum of force indicators by 50% of the initial values was 325 ± 3, 321 ± 4 and 300 ± 3 s ($n = 10$), and by 25%—300 ± 3, 296 ± 4 and 290 ± 4 s ($n = 10$) on days 1, 2 and 3 after the injury, respectively (Fig. 2), which was by 42–45% less relative to the damaged muscle in control

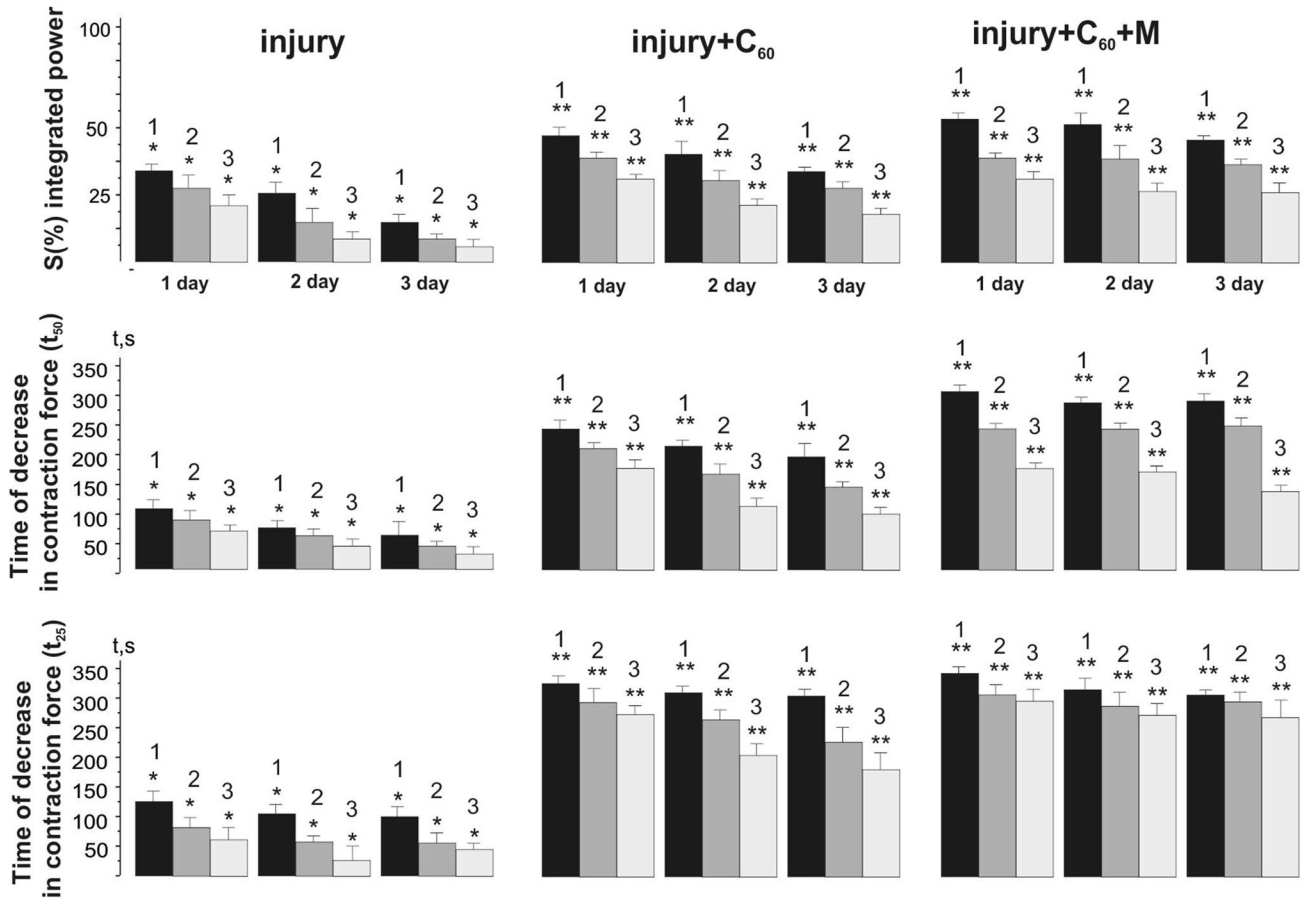

Fig. 2 Biomechanical parameters of muscle fatigue of posttraumatic *muscle soleus* after using therapeutic agents: S—integrated power of muscle contractions; t_{50} and t_{25}—the time to reach 50% and 25% of the initial muscle force of contraction, respectively; "injury + C_{60}" and "injury + C_{60} + M" indicate treatments by injections of C_{60} and C_{60} with menthol (M), respectively; 1, 2, 3—1, 2 and 3 sequential stimulation pool; a, b, c—power responses on days 1, 2 and 3 after muscle injury; $*p < 0.05$; $**p < 0.05$ relative to injury group

and by 19–21% less relative to the C_{60} therapy alone. It is important to note the significant difference in curves of force response after using C_{60} with menthol. A rapid drop in the maximum force response during the first 300 s (half of the muscle stimulation time) was up to 40% of the initial values. In the case of the C_{60} therapy, a gradual decrease of the maximum force of contraction is observed throughout the entire period of stimulation. A possible reason for this may be both a greater number of metabolic components in muscle fibers and stabilization of acidity in the intracellular space. It should also be noted that the use of the combined therapy did not eliminate the developing of fatigue processes in the muscle: the integrated muscle power decreased with each subsequent pool of stimulation (Fig. 2).

Next we analyzed the ability of the injured muscle to maintain maximum force during a 6 s period of muscle stimulation at 50 Hz. Figure 3 shows the curves of ten consecutive force responses of the injured *muscle soleus* 5 h after initiation of the injury. Decrease in the force of contraction during the first five consecutive contractions was replaced by almost complete muscle rigidity during the last stimulation pools. A rapid decrease in the force of contraction during the first stimulation and fluctuating force responses at the last stages of contraction are also notable.

Injections of C_{60} eliminated both the abrupt decrease in the contraction force and the fluctuation component of the contractile process (Figs. 3, 4). However, the integrated power of muscle contraction continued to decrease throughout the duration of stimulation with a slight increase of $12 \pm 3\%$ from the values of the injured untreated muscle. Menthol injections alone did not improve muscle dynamics studied with this protocol (data not shown). However, using combination of C_{60} and menthol resulted in an increase of the integrated power was by $29 \pm 2\%$ of the values of the injured muscle (Fig. 4). Reduction in the maximum force response was evident during each of ten consecutive contractions. C_{60} and menthol treatment increased this parameter during the first stages of contraction only. The decrease of time required for force to reach its maximum level was particularly notable after the combined therapy: from 1.3 s for

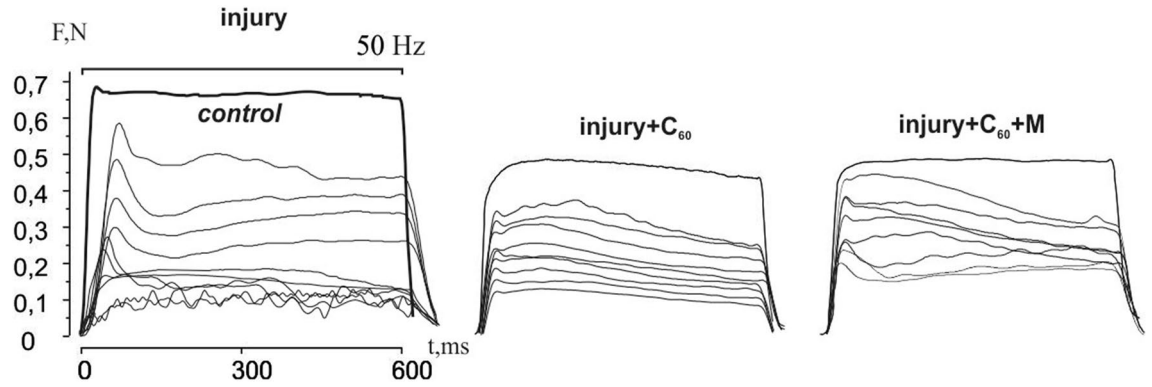

Fig. 3 Curves of 10 consecutive force responses of the injured *muscle soleus* to a stimulation signal with a frequency of 50 Hz of 6 s duration (without a relaxation period) in control (left panel) and after using the therapeutic agents 5 h after the initiation of muscle injury: "injury + C_{60}" and "injury + C_{60} + M" indicate treatments by injections of C_{60} and C_{60} with menthol (M), respectively

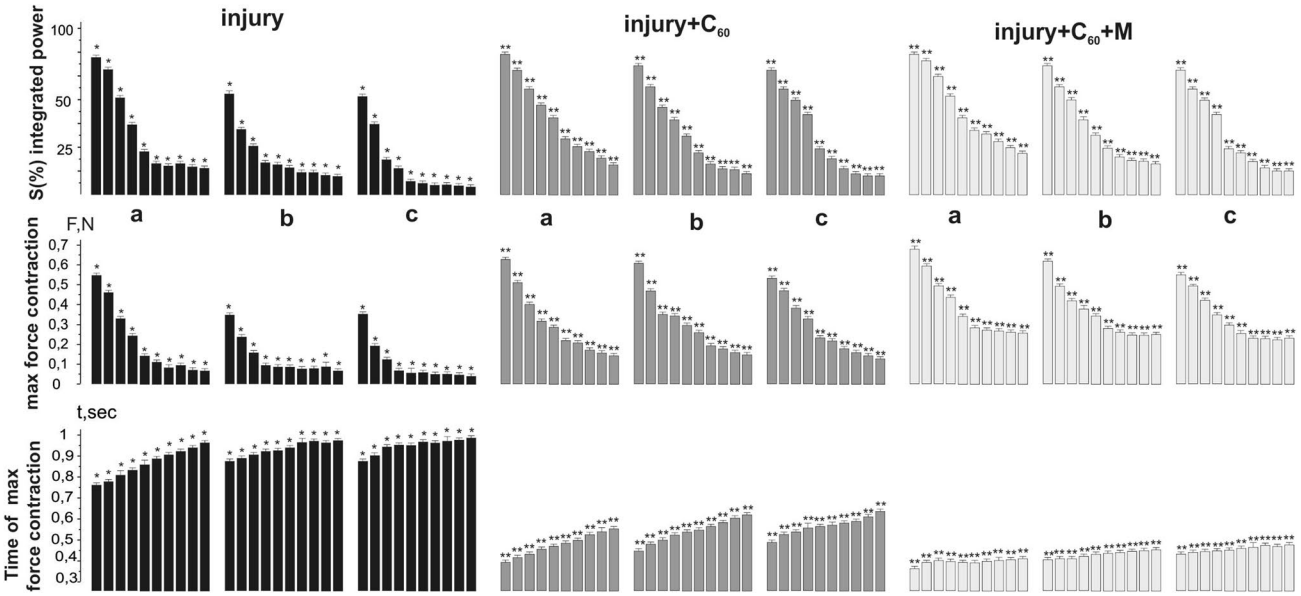

Fig. 4 Biomechanical parameters of 10 consecutive force responses of injured *muscle soleus* to stimulation at 50 Hz for a 6 s period (without a relaxation period) in control (traces marked "injury") and after using therapeutic agents: "injury + C_{60}" and "injury + C_{60} + M" indicate treatments by injections of C_{60} and C_{60} with menthol (M), respectively; a, b, c—curves of maximum strength responses on days 1, 2, and 3 after muscle injury, respectively; $*p < 0.05$; $**p < 0.05$ relative to the injury group

the injured muscle (control) to 0.5 s after the injection of C_{60} and 0.3 s for its combined injection with menthol (Fig. 4).

Analysis of these parameters on days 1, 2 and 3 after muscle injury showed a significant effect of the therapy on each of the studied biomechanical markers (Fig. 4). In the injured muscle, a decrease in the value of the mechanical response of the muscle to the stimulation was observed with an increase in the time after the injury. This is due to the progressive development of inflammatory processes after the initiation of the injury. After using of both C_{60} and C_{60} with menthol the increase of integrated power was by 55, 57, and 59% on days 1, 2 and 3 after the injury, respectively. The increase of maximum force response was by $42 \pm 2\%$, $46 \pm 3\%$, and $158 \pm 3\%$ ($n = 10$) on days 1, 2, and 3, respectively, after injection of C_{60} and by $62 \pm 1\%$, $66 \pm 2\%$ and $72 \pm 3\%$ on days 1, 2 and 3, respectively ($n = 10$) after injections of C_{60} with menthol.

Blood biochemical indicators of rats with injured muscle

Analysis of the selected biochemical blood markers indicative of the quality of skeletal muscle functioning, in particular changes in the levels of creatinine, creatine

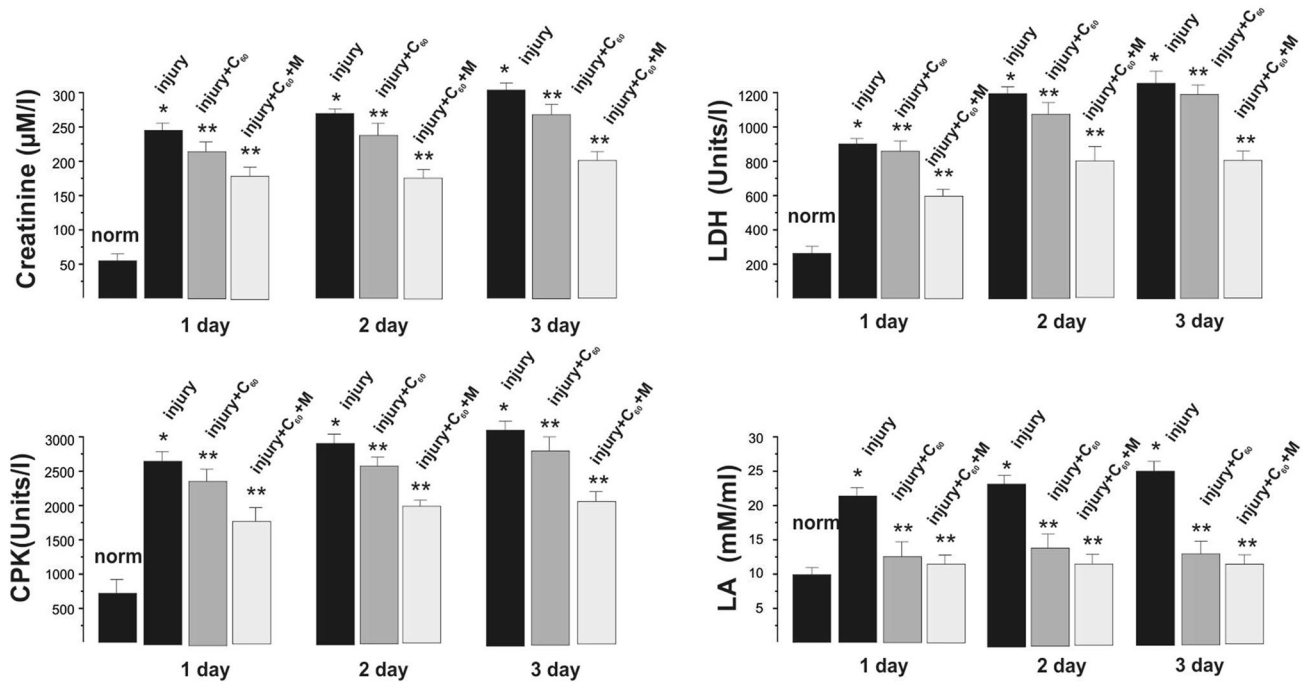

Fig. 5 Changes in the levels of creatinine, lactate dehydrogenase (LDH), creatine phosphokinase (CPK) and lactate (LA) in the blood of rats after muscle injury: "injury + C_{60}" and "injury + C_{60} + M" indicate treatments by injections of C_{60} and C_{60} with menthol (M), respectively; *$p < 0.05$; ** $p < 0.05$ relative to the injury group

phosphokinase (CPK), lactate (LA), and lactate dehydrogenase (LDH) in the blood of rats (Fig. 5), provides opportunities to assess the physiological changes in muscle and the therapeutic effect of the applied drugs on pathological processes. Previous studies have shown that all these markers have a pronounced tendency for elevation with increase in time after initiation of injury that indicates the super-intense for muscular system level of physiological work that is followed by the development of muscle fatigue.

One of the known markers of muscle fatigue is a change in the concentration of CPK, an enzyme involved in the energy supply, which catalyzes the transfer of a phosphate group from ATP to creatine with the formation of a high-energy compound creatine phosphate. After intensive functioning or mechanical damage of the muscles, the release of the enzyme from the cells and increase in CPK activity in the blood are observed. Increase in CPK blood fraction (Fig. 5) from 500 Units/l in control to 2380, 2422, and 2943 Units/l on days 1, 2, and 3 after the injury, respectively, is the result of destruction of myocyte walls caused by muscle injury (Gibala et al. 1995) with partial release of intramyocytic enzymes into the extracellular space. After injection of C_{60}FAS, the CPK level decreased by 9.3, 9.8, and 10.4% ($p < 0.05$) on days 1, 2, and 3, respectively. Combined administration of C_{60}FAS and menthol decreased the CPK level by 16.4, 16.8 and 17.5% ($p < 0.05$), which is evidence of the direct synergistic action of these agents. It is important to note that the use of menthol injections alone did not produce significant differences in any of the biochemical markers compared to the injured muscle.

Changes in LDH, the enzyme that catalyzes oxidation of lactic acid (the end product of glucose metabolism in cells during prolonged physical exertion), provide the means to assess the general state of functionality of the damaged muscle after its prolonged activity. Increase in LDH level from 200 Units/l (normal) to 860, 1180, and 1198 Units/l on days 1, 2, and 3, respectively after muscle injury (Fig. 5) is evidence of the development of significant dysfunction of the neuro-muscular system and, as consequence, the development of fatigue processes. After the injection of C_{60}FAS, LDH level decreased by 4.7, 5.2 and 5.1% ($p < 0.05$) on days 1, 2 and 3 after the injury, respectively. Combined administration of C_{60}FAS and menthol decreased the level of the enzyme by 29.7, 19.5 and 18.3% ($p < 0.05$), which is also significantly higher than with C_{60} therapy alone.

Changes in the level of creatinine, a product formed in muscles during the destruction of intramuscular structures, allowed us to assess the level of damage to myocytes. Figure 5 shows that this index increased from 50 μM/l in control to 240, 2562, and 297 μM/l on days 1, 2, and 3 after the injury, respectively. Injections of C_{60}, as in the analysis of its effect on the above described markers, did not show pronounced changes in creatinine content: creatinine level decreased by 5.3, 5.1 and 4.8% ($p < 0.05$) on days 1, 2, and

3 after the injury, respectively. However, combined administration of C_{60}FAS and menthol caused a marked decrease of creatinine level by 26.2, 27.4 and 26.7% ($p < 0.05$) that is significantly more than with C_{60} therapy alone. It is likely that a decrease in the creatinine fraction after combined therapy by C_{60}FAS and menthol is caused by the protective effect of menthol at the early stages of pathological process development by reducing inflammatory reactions, which then made it possible for C_{60} to show its antioxidant properties and protect the membranes of skeletal myocytes from nonspecific free radical damage by neutralisation of free radicals.

During the development of inflammatory reactions cascade after injury significant depletion of cellular energy substances, especially ATP, occurs, this leads to a disruption of homeostasis and a loss of ion gradients across the cell membranes. This, in turn, results in the accumulation of LA and H^+ ions, and, accordingly, acidification of the intra- and extracellular media (Hagberg et al. 1985). A decrease in ATP production suppresses the activity of the Na^+, K^+-ATPase, which leads to increase in the concentration of intracellular Na^+ and, as a consequence, intracellular Ca^{2+} (Ivanics et al. 2000). The increased content of K^+ ions causes a delay in the generation of the action potential and, accordingly, its propagation along the T-tubules (Jones 1996). Thus, ionic changes impair the muscle's ability to respond to electrical impulses, and lead to a decrease in muscle strength. Therefore, LA is an important marker for assessing the degree of performance of the injured muscle. Analysis of the LA level showed its increase after injury from 10 mM/ml in control to 23, 24, and 26 mM/ml on days 1, 2, and 3, respectively. The use of C_{60} therapy reduced its concentration by 48.5, 46.2 and 47.7% ($p < 0.05$) on days 1, 2, and 3, respectively. However, combined therapy using administration of both C_{60}FAS and menthol practically did not reduce the LA level: the decrease in its level was no more than 3% of the level with C_{60} therapy alone. Thus, under the conditions used for muscle injury development C_{60} is able to significantly reduce the LA level in the active muscle without additional activation of TRPM8 channels.

With the development of muscle pathology, a change in the level of antioxidants is an essential criterion that determines the level of physiological disbalance. The results of tests show the level of accumulation of the secondary products of lipid peroxidation in the blood of rats after induction of muscle injury (Fig. 6). The data obtained indicate an increased level of peroxidation and oxidative stress TBARS (thiobarbituric acid reactive substances) and H_2O_2 (hydrogen peroxide) after stimulation of the injured muscle. These markers increased on the second and third days after the initiation of injury and compared to the intact muscle were 235%, 308% and 423% ($p < 0.05$) for TBARS and 451%,

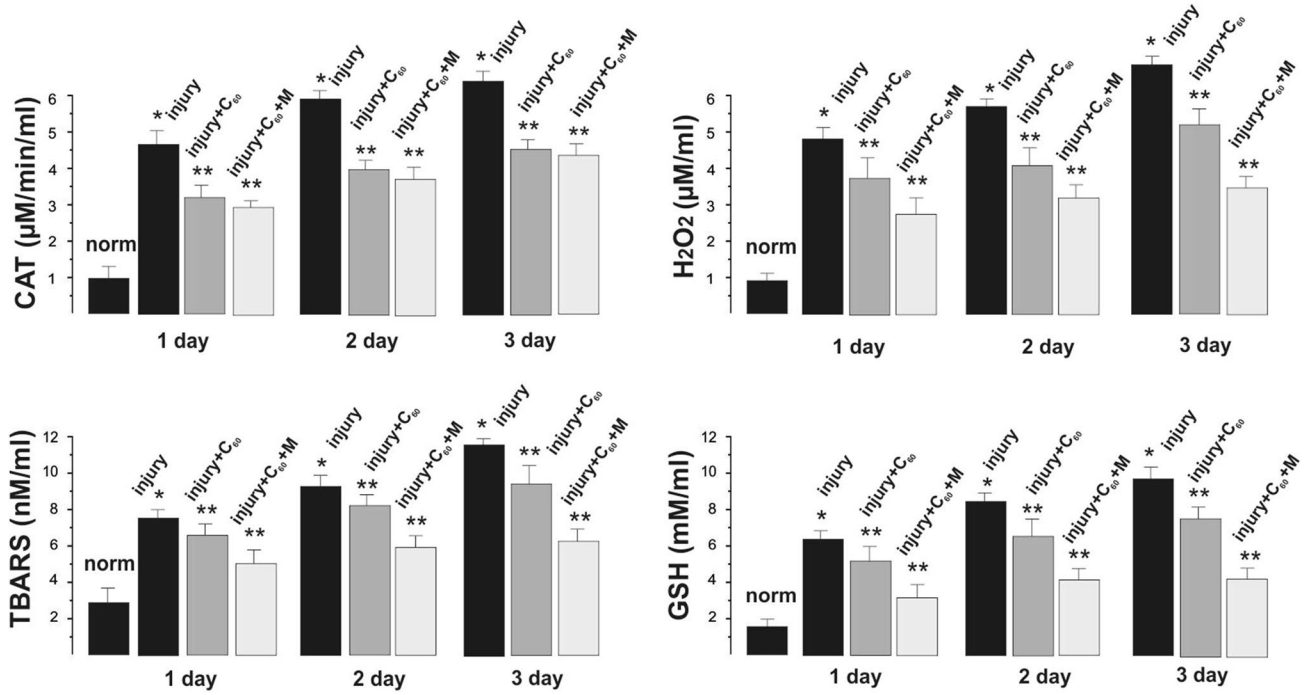

Fig. 6 Indicators of pro- and antioxidant balance in the blood of rats after induction of muscle injury (catalase (CAT) activity, hydrogen peroxide (H_2O_2), thiobarbituric acid reactive substances (TBARS), and reduced glutathione (GSH) concentrations): "injury + C_{60}" and "injury + C_{60} + M" indicate treatments by injections of C_{60} and C_{60} with menthol (M), respectively; *$p < 0.05$; **$p < 0.05$ relative to injury group

522% and 617% ($p < 0.05$) for H_2O_2 on days 1, 2, and 3, respectively. After administration of C_{60}FAS, TBARS concentration slightly decreased compared to the damaged muscle without therapy: 202%, 281%, and 367% ($p < 0.05$) on days 1, 2, and 3, respectively. Thus, the therapeutic effect of C_{60}FAS for this marker was no more than 11%. The decrease in the level of H_2O_2 after administration of C_{60}FAS was no more than 14% (Fig. 6).

The therapeutic effect of C_{60}FAS and menthol combined treatment on TBARS was 31%, 43% and 57% ($p < 0.05$), and on H_2O_2—47%, 49% and 51% ($p < 0.05$) on 1, 2 and 3 days, respectively, which was more than twofold higher than the therapeutic effect of C_{60}FAS alone. It should be noted that the most pronounced therapeutic effect was observed on the 3rd day after muscle injury. Analysis of the levels of the endogenous antioxidants showed a significant increase in GSH (reduced glutathione) levels—301%, 390% and 421% ($p < 0.05$) and activity of antiperoxide enzyme CAT (catalase)—471%, 527% and 578% ($p < 0.05$) on days 1, 2 and 3, respectively. GSH activity slightly decreased by 9%, 11% and 12% ($p < 0.05$), respectively, after administration of C_{60}FAS. The decrease in CAT indicators turned out to be more effective and was 14%, 18% and 19% ($p < 0.05$) on days 1, 2 and 3, respectively. GSH level decreased by almost 50% more than with C_{60} therapy after injections of C_{60}FAS and menthol: the therapeutic effect was 29%, 36% and 41% ($p < 0.05$) on days 1, 2 and 3, respectively. At the same time, the level of CAT remained practically unchanged compared to C_{60} therapy—the difference was no more than 2–3% (Fig. 6).

Significant differences in the severity of injury and the muscle group affected, as well as the nonspecificity of symptoms, complicate research aimed at identification of a suitable treatment for muscle injury. Therefore, it is important to understand the cellular processes inherent to this type of skeletal muscle injury and involved in their healing. The most important of these processes is inflammation as a consistent and sustained systemic response. The inflammatory response depends on two factors, specifically the degree of physical injury and the degree of muscle vascularization during injury. However, long-term anti-inflammatory treatment is not necessarily effective in accelerating healing, as indicated by various (Hurme et al. 1991). Due to a variety of ethical factors, studies of the inflammatory response during injury in humans are limited, but experimental animal models provide sufficient information to study muscle damage and regeneration. However, the methods currently used to induce mechanical damage vary considerably in terms of invasiveness, instruments used to induce injury, the muscle group selected for injury and their contractile status, and the effect on immune or cytokine responses. This complicates the interpretation of the results of such studies.

The early recovery phase of mechanical muscle injury is characterized by overlapping of inflammation process and development of secondary injury. Although neutrophil infiltration has been proposed as one of the reasons for the enhancement of inflammatory process, there is no clear evidence to support this statement. The main role in the initiation of inflammatory reactions is played by cascading, progressive increase in free radical components. Pathological inflammatory cascading processes that occur immediately after muscle injury are the source of free oxygen radicals and contribute to the intensification of lipid peroxidation processes (Davies et al. 1982). The presence of such metabolic products interferes with the adequate performance of muscle work and significantly increases the duration of their recovery period. A decrease in the concentration of these oxygen metabolites upon therapeutic administration of C_{60} should significantly improve the execution of motor commands of the central nervous system by the muscular system and contribute to a decrease in the level of pathological changes. In our opinion, the ability of C_{60} to effectively neutralize free radicals (Ferreira et al. 2018; Vereshchaka et al. 2018), is the main reason for the positive therapeutic results of the treatments described in this study.

Muscle tissue damage, as well as intense exercises, induce oxidative stress in skeletal muscle and therefore can alter the pro-antioxidant balance. Despite numerous studies have been done in this area, connections between free radicals, antioxidant enzymes, exercises, and skeletal muscle membrane injury remain controversial (Clanton et al. 1999; Ji 1995). These discrepancies may be related to differences in levels of tissue injuries, intensity and duration of muscle work, and muscle fiber type. Each type of muscle fibers has different metabolic characteristics and oxidative potential, as well as the ability to provide antioxidant protection (Ji et al. 1999). However, it remains indisputable that the presence of such metabolic products compromises the adequate performance of muscle work and significantly increases the duration of their recovery period.

It should be noted that during the development of inflammatory response of the muscle to traumatic injury in the primary alteration zone, the metabolic rate is reduced due to cellular dysfunctions, and in the secondary alteration zone it is increased due to the metabolism of carbohydrates (including glycolysis of polysaccharides). Oxygen consumption and carbon dioxide production are also increased; oxygen consumption exceeds the production of carbon dioxide, since oxidation does not always take place until the final formation of carbon dioxide (violation of the Krebs cycle). This leads to accumulation of under-oxidized metabolic products in the inflammation zone, which can be inactivated by C_{60}, optimizing the muscle recovery processes at this stage of the inflammatory process.

During intense physical activities, the flow of oxygen through muscle cells is significantly increased. A high level of oxygen uptake (up to 100-fold) can lead to excessive formation of reactive oxygen species (ROS) and initiate the destruction of functional myofibrils remaining after injury (Ji 1995). In our study, the increased amount of GSH in muscle (without and after therapeutic drugs administration) indicates a compensatory activation of the endogenous antioxidant system to the rapid inflammatory process initiated by muscle injury. Under our experimental conditions, this process is complicated by prolonged non-relaxation contractions of the injured muscle. Many studies have shown that under intense loads there is a significant decrease in the amount of reduced GSH paralleled by an increase in the concentration of its oxidative form (Leeuwenburgh et al. 1997). The described processes occur in the injured muscle with progressive dynamics for at least three days, after which time a recovery period begins.

An increase in H_2O_2 level in muscle injuries leads to increase of CAT activity, which performs a protective antioxidant role, catalyzing the decomposition of hydrogen peroxide into water and oxygen. These results are confirmed both by our studies and by studies carried out earlier in acute experiments on rats (Ji et al. 1992; Leeuwenburgh et al. 1995). At the same time, some studies indicated the absence of any changes in the concentration of CAT in muscles during their motor activity, which can be explained by the absence of decrease in this indicator with the applied therapeutic injections in comparison with other markers (Meydani et al. 1993).

After administration of C_{60} into the injured muscle during fatigue development, the CAT activity significantly decreased compared to fatigue alone. It can be assumed that C_{60}, by affecting the content and activity of endogenous antioxidants, prevent fatigue in actively contracting muscle and, thus, contribute to the maintenance of its normal physiological state (Prylutskyy et al. 2017). However, it should be noted that the level of pathological processes that arose at the first stages of the development of muscle injury exceeded the antioxidant capabilities of C_{60}.

The enhancement of the therapeutic effect of C_{60} in the presence of menthol can be explained by several mechanisms. Thus, studies have shown that menthol has a fast-acting, short-term effect of reducing blood flow, which reduces the level of inflammatory processes. In addition, a single 8 h application of an occlusive patch that contains 3% menthol to treat mild and moderate pain associated with mild and moderate muscle deformity in adult patients significantly alleviates it compared to patients who received placebo, which also affects the level of development of the subsequent inflammatory process (Higashi et al. 2010; Topp et al. 2011). It has been shown that with the internal use of menthol, there is an improvement in muscular performance, mediated by mechanisms associated with its thermal, ventilation, analgesic and stimulating properties (Stevens et al. 2017). Studying the local anaesthetic activity of menthol, the authors have documented its anaesthetic activity in in vivo and in vitro systems (Galeotti et al. 2001). It was also found that the local anaesthetic effect of menthol can be mediated by blockade of sodium channels, which is as effective as the local anaesthetic lidocaine (Haeseler et al. 2002). When an anaesthetic menthol balm is applied to the skin over contracting muscles, the pressor response to static muscle contractions is significantly reduced. This suggests that topical application of menthol has an effect on the responses caused by receptors located in the muscles (Ragan et al. 2004). These facts indicate the ability of menthol to promote the therapeutic properties of C_{60} as an antioxidant in severe pathological muscle injuries and, thus, to alleviate the inflammatory process. In our opinion, C_{60} can affect the activity of endogenous antioxidants, preventing the onset of dysfunction in the active muscle and, thus, maintaining it within the physiological norm during the entire process of muscle activation.

Moreover, we found that menthol can normalize vascular tone (Johnson et al. 2009), which explains, at least in part, the effectiveness of CryoDerm by its effect of TRPM8 cold receptors (https://www.cryoderm.com/index.php?p=392133). There is also accumulating evidence showing the important roles of several TRP subtypes, and notably TRPM8 receptor, in inflammatory and immune cells (Parenti et al. 2016). For example, it was shown that icilin, another selective TRPM8 agonist, reduced inflammation in mice with DSS-induced colitis, likely by inhibiting neuropeptide release (Ramachandran et al. 2013). Thus, TRPM8 agonists are currently considered as novel therapeutic strategies for alleviating intestinal inflammation (Chen et al. 2020). In addition, TRPM8 agonists could inhibit the synthesis of pro-inflammatory cytokines in both human lymphocytes and monocytes (Juergens et al. 2004).

Conclusion

Thus, it was found that injection of C_{60} at a concentration of 1 mg/kg into the damaged muscle of rat improved its contractile function by 25–28%. At the same time, the use of combined injections of C_{60} and menthol (at the concentration 1 mg/kg) improved this index by additional 27–39% and simultaneously stabilized the decrease in muscle strength observed throughout the experiment. A tendency towards a decrease in the indexes of the used biochemical parameters (creatinine, CPK, LA, LDH, TBARS, H_2O_2, GSH and CAT) by 10–15% were found with the therapeutic administration of C_{60}. With combined injections of C_{60} and menthol, these biochemical parameters decreased by an additional 17–24%.

The discovered positive changes in the biomechanical and biochemical parameters of the functioning of the injured skeletal muscle open up the prospect of using an aqueous solution of C_{60} in combination with menthol in low doses (1 mg/kg) as effective combinational therapy capable of correcting the pathological state of skeletal muscle following its mechanical injury.

Author contributions DN, TM and VS: biomechanical analysis. OV and KB: biochemical analysis. PS and UR: preparing of the samples. AZ and YP: coordination the research work, analysis of the data and preparing of the manuscript.

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Compliance with ethical standards

Conflict of interest Authors declare that they have no conflict of interest.

References

Amani H, Habibey R, Hajmiresmail SJ, Latifi S, Pazoki-Toroudi H, Akhavan O (2017) Antioxidant nanomaterials in advanced diagnoses and treatments of ischemia reperfusion injuries. J Mater Chem B 5:9452–9476. https://doi.org/10.1039/C7TB01689A

Chen L, Jia L, Xiujuan X, Ying L, Lv Y, Shanshan Q, Dandan L, Ronghua W, Xuzhi R, Jingxuan Z, Liang X, Xuanbin W, Jicheng C, Yonghong Z, Lanlan Z (2018) TRPM8 activation improves energy expenditure in skeletal muscle and exercise endurance in mice. Gene 641:111–116. https://doi.org/10.1016/j.gene.2017.10.045

Chen Y, Mu J, Zhu M, Mukherjee A, Zhang H (2020) Transient receptor potential channels and inflammatory bowel disease. Front Immunol 11:180. https://doi.org/10.3389/fimmu.2020.00180

Clanton TL, Zuo L, Klawitter P (1999) Oxidants and skeletal muscle function: physiologic and pathophysiologic implications. Proc Soc Exp Biol Med 222:253–262. https://doi.org/10.1046/j.1525-1373.1999.d01-142.x

Cuzzocrea S, Riley DP, Caputi AP, Salvemini D (2001) Antioxidant therapy: a new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. Pharmacol Rev 53:135–159

Davies KJ, Quintanilha AT, Brooks GA, Packer L (1982) Free radical and tissue damage produced by exercise. Biochem Biophys Res Commun 107:1198–1205. https://doi.org/10.1016/S0006-291X(82)80124-1

Eswaran SV (2018) Water soluble nanocarbon materials: a panacea for all? Curr Sci 114:1846–1850. https://doi.org/10.18520/cs/v114/i109/1846-1850

Ferreira CA, Ni D, Rosenkrans ZT, Cai W (2018) Scavenging of reactive oxygen and nitrogen species with nanomaterials. Nano Res 11:4955–4984. https://doi.org/10.1007/s12274-018-2092-y

Galeotti N, Ghelardini C, Mannelli L, Mazzanti G, Baghiroli L, Bartolini A (2001) Local anaesthetic activity of (+)- and (-)-menthol. Planta Med 67:174–176. https://doi.org/10.1055/s-2001-11515

Gibala MJ, MacDougall JD, Tarnopolsky MA, Stauber WT, Elorriaga A (1995) Changes in human skeletal muscle ultrastructure and force production after acute resistance exercise. J Appl Physiol 78:702–708. https://doi.org/10.1152/jappl.1995.78.2.702

Gillis DJ, Vellante A, Gallo JA (2020) Influence of menthol on recovery from exercise-induced muscle damage. J Strength Cond Res 34:451–462. https://doi.org/10.1519/JSC.0000000000002833

Gonchar OO, Maznychenko AV, Bulgakova NV, Vereshchaka IV, Tomiak T, Ritter U, Prylutskyy YI, Mankovska IM, Kostyukov AI (2018) C_{60} fullerene prevents restraint stress-induced oxidative disorders in rat tissues: possible involvement of the Nrf2/ARE-antioxidant pathway. Oxidative Med Cell Longevity. https://doi.org/10.1155/2018/2518676

Haeseler G, Maue D, Grosskreutz J, Bufler J, Nentwig B, Piepenbrock S, Dengler R, Leuwer M (2002) Voltage-dependent block of neuronal and skeletal muscle sodium channels by thymol and menthol. Eur J Anaesthesiol 19:571–579. https://doi.org/10.1017/s0265021502000923

Hagberg H (1985) Intracellular pH during ischemia in skeletal muscle: relationship to membrane potential, extracellular pH, tissue lactic acid and ATP. Pflügers Arch 404:342–347

Halenova T, Raksha N, Savchuk O, Ostapchenko L, Prylutskyy Y, Ritter U, Scharff P (2020) Evaluation of the biocompatibility of water-soluble pristine C_{60} fullerenes in rabbit. BioNanoSci 10:721–730. https://doi.org/10.1007/s12668-020-00762-w

Higashi Y, Kiuchi T, Furuta K (2010) Efficacy and safety profile of a topical methyl salicylate and menthol patch in adult patients with mild to moderate muscle strain: a randomized, double-blind, parallel-group, placebo-controlled, multicenter study. Clin Ther 32:34–43. https://doi.org/10.1016/j.clinthera.2010.01.016

Hurme T, Kalimo H, Lehto M, Järvinen M (1991) Healing of skeletal muscle injury: an ultrastructural and immunohistochemical study. Med Sci Sports Exerc 23:801–810

Isbary JW, Zeller H (1983) Experiences with Sportgel in the ambulatory treatment of athletic injuries. Fortschr Med 101:1351–1354

Ivanics T, Miklós Z, Ruttner Z, Bátkai S, Slaaf DW, Reneman RS, Tóth A, Ligeti L (2000) Ischemia/reperfusion-induced changes in intracellular free Ca^{2+} levels in rat skeletal muscle fibers - an *in vivo* study. Pflügers Arch 440:302–308. https://doi.org/10.1007/s004240000287

Ji LL (1995) Exercise sport science reviews: exercise and oxidative stress: role of the cellular antioxidant systems. Williams and Wilkins, Baltimore

Ji LL (1999) Antioxidants and oxidative stress in exercise. Proc Soc Exp Biol Med 222:283–292. https://doi.org/10.1046/j.1525-1373.1999.d01-145.x

Ji LL, Fu R (1992) Responses of glutathione system and antioxidant enzymes to exhaustive exercise and hydroperoxide. J Appl Physiol 72:549–554. https://doi.org/10.1152/jappl.1992.72.2.549

Johnson CD, Melanaphy D, Purse A, Stokesberry SA, Dickson P, Zholos AV (2009) Transient receptor potential melastatin 8 channel involvement in the regulation of vascular tone. Am J Physiol Heart Circ Physiol 296:H1868–H1877. https://doi.org/10.1152/ajpheart.01112/2008

Jones DA (1996) High- and low-frequency fatigue revisited. Acta Physiol Scand 156:265–270. https://doi.org/10.1046/j.1365-201X.1996.192000.x

Juergens UR, Engelen T, Racké K, Stöber M, Gillissen A, Vetter H (2004) Inhibitory activity of 1,8-cineol (eucalyptol) on cytokine production in cultured human lymphocytes and monocytes. Pulm Pharmacol Ther 17:281–287. https://doi.org/10.1016/j.pupt.2004.06.002

Leeuwenburgh C, Ji LL (1995) Glutathione depletion in rested and exercised mice: biochemical consequence and adaptation. Arch Biochem Biophys 316:941–949. https://doi.org/10.1006/abbi.1995.1125

Leeuwenburgh C, Hollander J, Leichtweis S, Griffiths M, Gore M, Ji LL (1997) Adaptations of glutathione antioxidant system to endurance training are tissue and muscle fiber specific.

Am J Physiol 272:R363–R369. https://doi.org/10.1152/ajpregu.1997.272.1.R.363

Matvienko TY, Zavodovskyi DA, Vulytska DA, Zay SY, Motuziuk OP, Bogutska KI, Nozdrenko DN, Sklyarov YP, Prylutskyy YI (2017) Water-soluble nanoscale C_{60} fullerenes as effective therapeutic means for prevention and correction of ischemic injury in skeletal muscle. Int J Physiol Pathophysiol 8:177–193. https://doi.org/10.1615/IntJPhysPathophys.v8.i2.80

Meydani M, Evans WJ (1993) Free radicals in aging: free radicals, exercise, and aging. CRC Press, Boca Raton

Nilius B, Owsianik G, Voets T, Peters JA (2007) Transient receptor potential cation channels in disease. Physiol Rev 87:165–217. https://doi.org/10.1152/physrev.00021.2006

Nozdrenko DM, Zavodovsky DO, Matvienko TY, Zay SY, Bogutska KI, Prylutskyy YI, Ritter U, Scharff P (2017) C_{60} fullerene as promising therapeutic agent for the prevention and correction of functioning skeletal muscle at ischemic injury. Nanoscale Res Lett 12:115. https://doi.org/10.1186/s11671-017-1876-4

Nozdrenko DN, Berehovyi SM, Nikitina NS, Stepanova LI, Beregova TV, Ostapchenko LI (2018) The influence of complex drug cocarnit on the nerve conduction velocity in nerve tibialis of rats with diabetic polyneuropathy. Biomed Res 29:3629–3634. https://doi.org/10.4066/biomedicalresearch.29-18-1055

Parenti A, De Logu F, Geppetti P, Benemei S (2016) What is the evidence for the role of TRP channels in inflammatory and immune cells? Br J Pharmacol 173:953–969. https://doi.org/10.1111/bhp.13392

Prilutski Y, Durov S, Bulavin L, Pogorelov V, Astashkin Y, Yashchuk V, Ogulchansky T, Buzaneva E, Andrievsky G (1998) Studyofstructureofcolloidalparticlesoffullerenesinwatersolution. Mol Cryst Liq Cryst 324:65–70. https://doi.org/10.1080/10587259808047135

Prylutska SV, Matyshevska OP, Grynyuk II, Prylutskyy YI, Ritter U, Scharff P (2007) Biological effects of C_{60} fullerenes *in vitro* and in a model system. Mol Cryst Liq Cryst 468:265–274. https://doi.org/10.1080/15421400701230105

Prylutska SV, Grebinyk AG, Lynchak OV, Byelinska IV, Cherepanov VV, Tauscher E, Matyshevska OP, Prylutskyy YuI, Rybalchenko VK, Ritter U, Frohme M (2019) *In vitro* and *in vivo* toxicity of pristine C_{60} fullerene aqueous colloid solution. Fullerenes Nanotub Carb Nanostr 27:715–728. https://doi.org/10.1080/1536383X.2019.1634055

Prylutskyy YI, Vereshchaka IV, Maznychenko AV, Bulgakova NV, Gonchar OO, Kyzyma OA, Ritter U, Scharff P, Tomiak T, Nozdrenko DM, Mischenko IV, Kostyukov AI (2017) C_{60} fullerene as promising therapeutic agent for correcting and preventing skeletal muscle fatigue. J Nanobiotechnol 15:8. https://doi.org/10.1186/s12951-016-0246-1

Ragan BG, Nelson AJ, Foreman JH, Bell GW, Iwamoto GA (2004) Effects of a menthol-based analgesic balm on pressor responses evoked from muscle afferents in cats. Am J Vet Res 65:1204–1210. https://doi.org/10.2460/ajvr.2004.65.1204

Ramachandran R, Hyun E, Zhao L, Lapointe T, Chapman K, Hirota C, Ghosh S, McKemy D, Vergnolle N, Beck P, Altier C, Hollenberg M (2013) TRPM8 activation attenuates inflammatory responses in mouse models of colitis. Proc Natl Acad Sci USA 11:7476–7481. https://doi.org/10.1073/pnas.1217431110

Ritter U, Prylutskyy YI, Evstigneev MP, Davidenko NA, Cherepanov VV, Senenko AI, Marchenko OA, Naumovets AG (2015) Structural features of highly stable reproducible C_{60} fullerene aqueous colloid solution probed by various techniques. Fullerenes Nanotubes Carbon Nanostruct 23:530–534. https://doi.org/10.1080/1536383X.2013.870900

Scharff P, Carta-Abelmann L, Siegmund C, Matyshevska OP, Prylutska SV, Koval TV, Golub AA, Yashchuk VM, Kushnir KM, Prylutskyy YuI (2004) Effect of X-ray and UV irradiation of the C_{60} fullerene aqueous solution on biological samples. Carbon 42:1199–1201. https://doi.org/10.1016/j.carbon.2003.12.055

Souza Jd, Gottfried C (2013) Muscle injury: review of experimental models. J Electromyogr Kinesiol 23:1253–1260. https://doi.org/10.1016/j.jelekin.2013.07.009

Stevens CJ, Best R (2017) Menthol: a fresh ergogenic aid for athletic performance. Sports Med 47:1035–1042. https://doi.org/10.1007/s40279-016-0652-4

Topp R, Winchester L, Mink AM, Kaufman JS, Jacks DE (2011) Comparison of the effects of ice and 3.5% menthol gel on blood flow and muscle strength of the lower arm. J Sport Rehabil 20:355–366. https://doi.org/10.1123/jsr.20.3.355

Vereshchaka IV, Bulgakova NV, Maznychenko AV, Gonchar OO, Prylutskyy YI, Ritter U, Moska W, Tomiak T, Nozdrenko DM, Mishchenko IV, Kostyukov AI (2018) C_{60} fullerenes diminish the muscle fatigue in rats comparable to N-acetylcysteine or β-alanine. Front Physiol 9:517. https://doi.org/10.3389/fphys.2018.00517

Zholos A, Johnson C, Burdyga T, Melanaphy D (2011) TRPM channels in the vasculature. Adv Exp Med Biol 704:707–729. https://doi.org/10.1007/978-94-007-0265-3_37