Effect of low-level laser (Ga-Al-As 655 nm) on skeletal muscle fatigue induced by electrical stimulation in rats

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Lopes-Martins, Rodrigo Álvaro B., Rodrigo Labat Marcos, Patrícia Sardinha Leonardo, Antônio Carlos Prianti, Jr., Marcelo Nicolas Muscará, Flavio Aimbire, Lúcio Frigo, Vegard V. Iversen, and Jan Magnus Bjordal. Effect of low-level laser (Ga-Al-As 655 nm) on skeletal muscle fatigue induced by electrical stimulation in rats. J Appl Physiol 101: 283–288, 2006. First published April 20, 2006; doi:10.1152/japplphysiol.01318.2005.—We investigated whether low-level laser therapy (LLLT) can reduce muscular fatigue during tetanic contractions in rats. Thirty-two male Wistar rats were divided into four groups receiving either one of three different LLLT doses (0.5, 1.0, and 2.5 J/cm2) or a no-treatment control group. Electrical stimulation was used to induce six tetanic muscle contractions in the tibial anterior muscle. Contractions were stopped when the muscle force fell to 50% of the initial value for each contraction (T50%). There was no significant difference between the 2.5 J/cm2 laser-irradiated group and the control group in mean T50% values. Laser-irradiated groups (0.5 and 1.0 J/cm2) had significantly longer T50% values than the control group. The relative peak force for the sixth contraction in the laser-irradiated groups were significantly higher at 92.2% (SD 12.6) for 0.5 J/cm2 than for the control group [50% (SD 15)]. Laser groups receiving 0.5 and 1.0 J/cm2 showed significant increases in mean performed work compared with both the control group and their first contraction values. Muscle damage was indirectly measured by creatine kinase levels in plasma. A distinct dose-response pattern was found in which 1.0 and 2.5 J/cm2 LLLT groups had significantly lower creatine kinase levels than the 0.5 J/cm2 LLLT group and the control group. We conclude that LLLT doses of 0.5 and 1.0 J/cm2 can prevent development of muscular fatigue in rats during repeated tetanic contractions.

laser therapy; skeletal muscle; electrical stimulation; rats; fatigue

FATIGUE IS A COMMON EXPERIENCE in daily life, although the cellular and physiological mechanisms involved are not fully understood. The main features of muscular fatigue are decreased muscle strength, impaired motor control, and subsequently muscular pain. Several studies have investigated how fatigue develops during different types of exercise to elucidate the process and mechanisms involved. Decreased muscle strength has been reported after fatigue in different parts of the human body (7, 24, 39). Physical fitness is a major factor in preventing fatigue, but within common physiological limits anyone may experience a state of neuromuscular fatigue if the time period is long enough (16). Whether age-dependent changes in physical performance result from alterations in muscle fibers, neuromuscular junction, or peripheral nerve and/or central motor neurons has been difficult to ascertain in vivo (19). Exercise-induced muscle injury in humans may occur after strenuous exercises, particularly if the exercises involve a large amount of eccentric contractions (9). In patients with chronic neck pain of muscular origin, both early onset of increased electromyographic activity and a lack of postexercise vasodilatation have been observed compared with healthy controls (28).

There are several definitions for muscular fatigue, and one of them is the incapacity to maintain force by muscular contraction for a period of time. From an athlete’s perspective, Vollestad’s (49) definition of fatigue, consisting of any exercise-induced reduction in the maximal capacity to generate muscle force, might be appropriate. Verburg et al. (48) also state that fatigue develops during maximal or high-intensity exercise and during prolonged submaximal exercises, as evidenced by a decline in the force-generating capacity during maximal (test) contractions. It has been shown that a considerable component of fatigue is due to processes on the surroundings of the muscular junction (4, 31, 41), i.e., inhibition of neural excitation-muscular contraction coupling (1, 18, 43). Most hypotheses for the mechanism of this type of fatigue are based on changes in muscle function and local biochemical changes, which occur during high-intensity exercise or tetanic stimulation (48).

On the other hand, it is well established that altering O2 delivery to the contracting skeletal muscle affects human performance. In this respect, reducing O2 supply (hypoxia) will increase the rate of muscle fatigue, whereas increasing O2 supply (hyperoxia) will reduce the rate of fatigue (23).

Numerous studies have used experimental models involving maximal voluntary contractions, tetanic stimulation, and ischemic conditions (5, 36, 50) to study skeletal muscle fatigue. In experimental models, it has been demonstrated that a reduced force generation follows intense, sustained voluntary contrac—

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tions and tetanic electrical stimulation of animal muscles in vitro (4, 13, 15).

Several tools have been used to prevent muscle fatigue in healthy athletic subjects as well as in pathological conditions such as nutritional supplements (14, 51, 52), fluid reposition (10, 27), vitamin C (45), creatine (33), and arginine (32, 38).

Low-level laser therapy (LLLT) is a novel therapy form that has been used to treat muscular pain, but the biological mechanisms behind observed beneficial results in clinical trials remain unclear (8). LLLT may be administered with different wavelengths of the visible and near-infrared spectra including HeNe (632.8 nm), Ga-Al-As (805 or 650 nm), and Ga-As (904 nm) (34). LLLT has been shown to reduce the duration of acute inflammation (35) and accelerate tissue repair in tendon and muscle injuries (3). Positive effects from LLLT may be related to modulation of the oxidative chain, and a recent review concluded that LLLT also increased cellular redox activity (29). Stimulatory effects from LLLT have been observed for mitochondrial activity in cell cultures (53), and in hypoxic animal tissue (54) of diabetic mice. Marked increases in arteriolar and collateral blood flow have also been observed in injured tissue after LLLT (25, 30). Delayed onset muscle soreness (DOMS) is often observed after eccentric exercises. DOMS is manifested clinically through transient muscle pain and impaired maximal muscle strength. Physiological manifestation may be increased levels of creatine kinase (CK), which is considered to be an indirect sign of widespread microtraumas inside the muscle. It has been shown that the anti-inflammatory drug diclofenac reduces minor muscle damage and CK levels in DOMS. In clinical settings, positive effects have also been observed in chronic muscular pain conditions. For instance, painful fibromyalgia is associated with impaired local circulation (26), and this disorder also seems to benefit from LLLT (21).

In this perspective, it is a clear need to investigate whether LLLT can prevent hypoxia and muscular fatigue. We decided to use the experimental model of electrical nerve stimulation to induce fatigue by tetanic tibial muscle contraction in rats and test whether LLLT could prevent the anticipated reduction of muscle force or the anticipated increase in CK levels.

METHODS

All experiments were carried out in accordance with the guidelines of Vale do Paraíba University for human and animal care, protocol no. 045/2003/CEP.

Materials

The experiments were carried out on male Wistar rats weighing between 150 and 200 g, with food and water “ad libitum” provided by Central Animal House of the Research and Development Department of Vale do Paraíba University (UNIVAP). All rats were placed in a box, and 32 animals were randomly divided into four groups of eight animals as follows: 1) control group nonirradiated; 2) treatment group receiving LLLT administered with 1.0 J/cm²; 3) treatment group receiving LLLT administered with 2.5 J/cm²; and 4) treatment group receiving LLLT administered with 5.0 J/cm².

Experimental Procedure

Animal preparation. Rats were anesthetized intraperitoneally with pentobarbitone sodium (40 mg/kg), and the former tibial muscle and tibial nerve were both isolated and immediately fixed to a surgical table. In the insertion region next to the plantar region from metatarsus, the muscle and tendon were connected to an isometrical transducer (Ugo Basile; Vareze, Italy) and the nerve was connected to a bipolar electrode. The muscle was submitted to a constant tension of 0.1 N, which was achieved by titrating electrical stimulation intensity with a pulse frequency of 0.2 Hz.

Electrical stimulation for induction of muscular fatigue. The electrical stimulation was administered with an intensity of 6–7 V and pulses of 2-ms duration. To induce a tetanic contraction, the frequency was raised from 0.2 to 60 Hz and kept there until the muscular force fell to 50% of its value at the onset of that particular contraction (Fig. 1).

This procedure was repeated six times for every 10 min during the following 60 min. The muscle force was measured by a dynamometer in Newtons (UGO Basile Gemini 7070 Physiograph). To avoid tissue death, electrical stimulation was kept constant only until the tension from muscular contraction declined to 50% under the maximum recording. The muscle was then rested until the next contraction commenced 10 min after the start of the previously induced contraction.

Laser irradiation. A diode laser of the InGaAIP type (Dermolaser AD2040) with a continuous output power of 2.5 mW and wavelength of 655 nm (visible red) was used. The optical power was calibrated using a Newport multifunction optical meter model 1835C. The stability of laser during the laser irradiation was measured collecting light with a partial reflect (4%). The spot size was 0.08 cm², and the laser illumination spot was placed in contact with the central part of dissected tibial muscle belly. Irradiated animals received irradiation in this single point once before the first muscle contraction. Irradiation lasted 32, 80, and 160 s, respectively, with a fixed power density of 31.25 mW/cm². The total delivered energy for irradiated groups were 0.08, 0.2, and 0.4 J, respectively.

Outcomes

Muscular fatigue was defined by three parameters: 1) the maximal force elicited at the onset of each of the six electrically induced tetanic contractions; 2) the time taken for an electrically induced tetanic contraction to have its force reduced to 50% of the value at the onset of that particular contraction; and 3) the work performed by each electrically induced tetanic contraction [power (Watts) = force (Newton) × time (seconds)].

Muscle damage was indirectly measured by the levels of CK. CK levels were measured from blood samples collected in vivo during the experiment between the electrical stimulation sessions. The blood samples were collected from each animal and then analyzed.
Statistical Analysis

The obtained data were first plotted for analysis of normal distribution, and statistical analysis was then performed with parametric tests if the data were normally distributed. The statistical level of significance was set at $P < 0.05$. After confirmation, obtained data were tested statistically by an ANOVA. The mean value and its standard error (SE) were calculated, and differences between control group data and the irradiated group data were tested statistically with Student’s $t$-test.

RESULTS

In all the contractions, a typical nonlinear decrease of force could be seen over time (Fig. 2).

In the control group, the mean peak contraction force decreased in a successive but nonlinear manner to 50% (SD 15) of the first mean peak contraction force in the sixth contraction. For the laser-irradiated groups, the mean peak contraction force was significantly higher at 92.2% (SD 12.6) for 0.5 J/cm$^2$, 83.2% (SD 20.5) for 1.0 J/cm$^2$, and 82.9% (SD 18.3) for 2.5 J/cm$^2$ for the sixth contraction (Fig. 2).

In the control group, the mean time to 50% decrease of the maximal force of each particular contraction did not change significantly over time and remained between 92% (SD 40.4) and 110% (SD 12.5) of the first contraction. For the laser-irradiated groups, the mean time to 50% decrease of the maximal force showed different patterns for different doses. There was no statistically significant difference between the change in time to 50% decrease of maximal force in the laser group receiving a dose of 2.5 J/cm$^2$ and the control group for any of the six contractions. The laser group receiving 0.5 J/cm$^2$ had a significantly longer time to 50% decrease in maximal force of the second, third, and fourth contractions than the control group, whereas the laser group receiving 1.0 J/cm$^2$ had significantly longer times to 50% decrease in maximal force for all contractions than did the control group (Fig. 3).

In the control group, the mean work elicited before a 50% decrease of the maximal force of each contraction dropped sharply from the first to the second contraction (67% (SD 6.4)) and decreased less in a linear manner from the third to sixth contraction. For all laser-irradiated groups, the mean work elicited was significantly higher than the control group for every single contraction. However, this was significantly more pronounced in the 0.5 and 1.0 J/cm$^2$ groups than in the 2.5 J/cm$^2$ group (Fig. 4).

We measured CK levels by spectrophotometry before the first tetanic contraction and after the third and sixth contraction (Fig. 6). In the control group, we found a linear reduction of CK levels, which were reduced by 15% after the third contraction and 30% after the sixth contraction. For the laser groups, we found a significantly higher reduction of CK levels at all time points except after the sixth contraction for the 0.5 J/cm$^2$ group. The laser dos-response patterns for CK level reductions differed from the other outcomes in the sense that the lowest laser dosage also induced the smallest reductions of CK levels. The largest reduction of 80% of CK levels were seen with a

Fig. 2. Typical record of rat tibial skeletal muscle tetanic contraction induced by electrical stimulation. A, peak force of contraction in Newtons; B, time to decrease to 50% of maximal contraction. The area under the curve represents our parameter for performed workload.

Fig. 3. Percent muscular force in Newtons over time for a tetanic contraction induced by electrical nerve stimulation. The initial peak force was taken as 100%. Each contraction was induced every 10 min in a total of 6 tetanical contractions both for control and irradiated animals. $*P < 0.05$.

Fig. 4. Graph showing group mean times with SE for contraction force to be reduced to 50% of the maximal force achieved from the onset of each of 6 maximal muscular contractions. The initial time was taken as 100%. The treatment group receiving doses of 1.0 J/cm$^2$ were less fatigued and able to maintain >50% muscle force significantly longer than the control group at contractions 2 through 6. $*P < 0.05$. 
dose of 1.0 J/cm² after the sixth contraction, whereas the high laser dose (2.5 J/cm²) induced significantly better CK level reductions than the low-dose laser group (0.5 J/cm²) (Fig. 5).

**DISCUSSION**

In this trial, we have demonstrated that LLLT can postpone the fatigue response to repeated tetanic contractions in skeletal muscles. Our results also show some differences in effects between the different doses used. Within the common therapeutic dose range between 0.5 and 2.5 J/cm², LLLT provided superior muscular performance by generating consistently higher maximal force for all three doses involved. However, the highest LLLT dose was not superior to controls when only the times to half values of maximal force were considered. This indicates that some of the beneficial effects on cellular metabolism may be lost if LLLT doses are too high. Although not significantly different from controls, the highest dose group was clearly superior to controls in reducing postexercise CK levels (Fig. 6). A preventive LLLT effect on inflammatory reactions to the strenuous exercises induced in the experiment is suggested from the two highest doses of 1 and 2.5 J/cm². The observed dose-response pattern for LLLT is familiar and has been observed previously in reviews of connective tissue metabolism and inflammation. Indeed, fibroblast cell metabolism is stimulated and collagen production increased after LLLT with a dose range that is slightly lower than what is required to suppress inflammatory reactions (6, 37, 40, 47). The ability to enhance cellular survival in hypoxic tissue has previously been demonstrated in LLLT-treated dogs with myocardial infarcts (34). Our observations provide an explanation for the beneficial LLLT effects seen in chronic muscular pain syndromes (22). However, a few considerations for optimizing clinical use need mentioning. First, the doses we used here are “in situ” doses, because the muscles were dissected and the animal skin was removed. In a clinical setting, increased doses are needed to penetrate human skin (44). Second, the irradiated muscle in our experiment was only 1 mm thick, and thus most of the rat tibial muscle received an adequate LLLT dose. Although LLLT can exhibit positive effects on hemoglobin and blood cells (42), particularly hemoglobin absorbs laser light and may hamper an even distribution of the LLLT dose (46). In larger human skeletal muscles, it may also be difficult to distribute adequate LLLT doses throughout the muscle belly.

Few clinical studies have investigated the effects of LLLT in DOMS, but two studies found no significant effects from doses of combined laser and light-emitting diode therapy by red 660-nm and infrared 850- and 950-nm wavelengths with 11 and 31 J/cm², respectively (11, 12). There are several possibilities for the poor results in these studies. Either the light-emitting diode and laser combination or the combination of different wavelengths or doses above 10 J/cm² may have caused the poor results. In addition, they only assessed pain and range of motion and did not measure the ability to elicit force or perform work. In sports, quick and adequate restoration of physiological processes after strenuous training or competition is important, and several options exist to achieve this. To our knowledge, LLLT has not been used as an adjunct to restore bodily processes after sports.

In other areas, such as chronic muscle pain syndromes, clinical studies have shown that doses of 2 J/cm² with monochromatic 904-nm pulsed laser have achieved good results (21, 22). Several treatment options are available for the treatment of chronic muscle pain, but currently none seem to provide the ultimate balance between benefits and harms. In addition, some disorders, like chronic neck muscle pain and fibromyalgia, may be recalcitrant, and no single treatment has provided convincing results. Common treatments for muscle pain are often associated with transient painful perceptions like massage and acupuncture, and pharmaceutical muscle relaxants may cause addiction and inflict poorer prognosis in low back pain (17). Fibromyalgia is a chronic condition where fatigue and hypoxia seem to play an important part (26). Although exercise therapy...
has been used with some long-term success in fibromyalgia, the level of intensity must be increased slowly to avoid episodes of increased pain and setback (20). Exercise-induced fatigue is often associated with painful reactions in fibromyalgia patients, and LLLT may have potential in reducing their postexercise pain. Similarly, in chronic neck muscle pain, the normal increase in blood flow during and after strenuous work seems disturbed (28) and may cause hypoxia and inefficient recruitment of muscle fiber contractions.

In this perspective, there is clearly room for a risk-free and effective alternative, and LLLT seems a likely contender. Future clinical studies are needed to systematically elucidate the possible mechanisms behind LLLT in muscle fatigue and muscle pain and the optimal administration and dosage of LLLT.

In conclusion, this animal study showed that LLLT could reduce the debilitating influence of local fatigue on muscle force and possibly reduce muscle damage after strenuous exercises in a dose-dependent manner. Further studies in clinical settings are warranted.

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