

MLS LASER TREATMENT: A TOOL TO COUNTERACT MICROGRAVITY-INDUCED MUSCLE ATROPHY

Monici, M.

ASAcampus JL, ASA Res. Div., ASA & Dip. Clin. Physiopat., Florence Univ., Italy.

It is well known that muscle atrophy, together with bone loss and impairment of immune system, is a major problem in space missions and countermeasures are needed to prevent disuse atrophy and favour muscle recovery.

Studies on microgravity-induced muscle atrophy demonstrated that it is associated with a shift in substrate utilization from fat to glucose, altered mitochondrial function, increase in ATP consumption per force-time-integral, indicating a reduced metabolic efficiency.

Lasertherapy is already used in sports medicine to accelerate muscle recovery after exercise and prevent damages produced by metabolic disturbances and inflammatory reactions after heavy exercise (Leal Junior et al., 2009).

The aim of the research we present was to get insights on possible benefits deriving from the application of an advanced laser system to counteract deficits of muscle energy metabolism.

The laser source was a Multiwave Locked System (MLS), which combines continuous/pulsed emissions at 808 nm and 904 nm., respectively.

We used the C2C12 cell line, a myoblast model, to study the effect of MLS treatment on cell energy metabolism. Cells were treated 8 min daily for 4 consecutive days (frequency 1500 Hz, energy 198,2 J, fluence 2J/cm² in 10 sec). Intracellular redox state was evaluated by autofluorescence (AF) microscopy (Schneckenburger and König, 1992). Glucose 6-P dehydrogenase (G6PDH), isocitrate dehydrogenase (ICDH), malate dehydrogenase (MDH), glyceraldehyde 3-P dehydrogenase (GAPDH), hexokinase, enolase, pyruvate kinase (PK), triosephosphate isomerase (TIM), hydroxyacylCoA dehydrogenase and lactate dehydrogenase (LDH) activities were determined continuously, following NAD(P) reduction or NADH oxidation at 340nm, using an UV-2100 spectrophotometer (Shimadzu, Columbia, MD). To investigate the different protein expression in C2C12 cells and controls a comparative proteome analysis was performed. Proteins were extracted, resolved by 2-D SDS-PAGE and the resulting colloidal coomassie stained electropherograms were analyzed using the Image Master 2D Platinum software. Identification of interesting proteins was carried out by MALDI-TOF MS.

Finally, cell morphology, cytoskeleton organization and expression of MyoD, an early marker of myogenic commitment, were analyzed by immunofluorescence microscopy and image analysis.

After laser treatments cell AF decreased of about 40%. The activity of MDH, LDH, PK, GAPDH and TIM increased. Proteome analysis pointed out about 150 quantitative variations between control and laser treated cells, while about 20 and 50 spots were exclusively detected in control and laser treated cells, respectively. Immunofluorescence microscopy showed an increase of about 20% in MyoD expression, rearrangement of actin microfilaments and microtubules.

Increase in activity of key enzymes and changes in the turnover of reduced/oxidized forms of pyridinic coenzymes and flavins demonstrate that MLS treatment strongly affect cell energy metabolism.

Proteome analysis, cytoskeleton rearrangement and MyoD expression suggest that the treatment induces important changes in protein expression and cell morphology and could promote myogenic commitment.

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