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Letter to the Editor

High-Tech Helps to Estimate Cellular Mechanisms of Low Power Laser Therapy

Clinical endpoints of low power laser therapy are initiated by a sequence of biochemical events at the molecular and cellular levels. In spite of years of successful treatment of variety of conditions including arthritis and soft tissue injuries (recent review: [3]) and laboratory work (reviews: [4,5]), the action mechanism of this therapeutic modality is not clearly understood. This is one reason why there remains a considerable amount of ignorance and skepticism concerning clinical efficiacy of low power laser therapy (photobiomodulation).

I would like to draw the attention of readers *of "Lasers in Surgery and Medicine"* towards two recent studies that were not published in laser medicine community journals. These studies investigate cellular mechanisms of photobiomodulation using most advanced experimental techniques, cDNA microarray technology [1] and single cell confocal microscopy [2].

In the work [I], gene expression profiles of human fibroblasts revealed that 111 genes were regulated by the irradiation of cells at 628 nm in parallel with the increase of proliferation. These 111 genes belong to 10 functional categories, 7 of which directly or indirectly play roles in the enhancement of cell proliferation and the suppression of apoptosis.

Biochips and microarrays are revolutionary new technologies enabling an entirely new approach to biomedical research [6]. Until now, researchers study one or a few genes at a time. With whole-genome sequence and new automated, high-throughput microarray and biochip technologies, a medical problem (in our case—cellular mechanisms of photobiomodulation) can be studied systematically, simultaneously, and on a large scale. The questions how hundreds and thousands of genes and proteins work together and how they are regulated in interconnected cellular network can be answered with the help of these new technologies. High-density microarray technology can simultaneously analyze the expression of thousands of different genes. Work [1] is only a beginning in use of this technology and I am sure that we will hear much more in the near future. The experimental results of this first attempt to use microarrays for investigation of low power laser cellular mechanisms corroborate in many aspects the data gathered by the old biochemical techniques [4,5]. But it also evidences that the cellular mechanisms of photobiomodulation are much more complicated than previously thought [4,5].

In the work [2], confocal laser scanning microscopy was used for irradiation (λ=647 nm) of human fibroblasts. Observation of the same area of interest allowed the imaging and quantifying the effects at the single cell level in real time scales. After laser irradiation, a gradual alkalization of the cytosolic pH (increase in pH;) with a maximum value at 6 min occurred; after 15 min, the pH, returned to its resting level. Laser irradiation was found to trigger recurrent spikes in the intracellular calcium concentration. Mitochondrial membrane potential was increased at 2 min after laser irradiation and reached 30% of its basal level. Also, an increase in generation of reactive oxygen species (ROS) was monitored.

Quantitative confocal microscopy imaging systems are already in use for some years but to my best knowledge the work [2] is the first one that uses this method for direct measurement of responses of single cells to low power laser light. In this work, some of the key reactions, which were suggested earlier to occur (jump in ApH,, ROS generation, Ca^{24}" oscillation) [4,5], are measured in real time scale. Important finding in work [2] is that some cells, even in the same culture coverslips, responded to a lesser degree to the laser stimulation. This is not surprising because cells grown in exactly the same conditions are not homogeneous in sense of intracellular homeostatic parameters at the moment of irradiation. The work [2] gives a quantified and measured in real time scale answer why the photobiomodulation effects are not always of equal strength.

I hope that two recent studies [1,2], the authors of which use most advanced contemporary technologies, open a new page in investigation of cellular mechanisms of low power laser therapy and the improvement of understanding of clinical usefulness of this therapeutic modality.

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Low level light (or laser) therapy (LLLT) has been used for more than forty years to promote healing, reduce pain and inflammation, and prevent tissue death [1,2]. Despite many basic and clinical reports, the therapy remains controversial largely due to uncertain-ties about the fundamental molecular and cellular mechanisms responsible for transducing signals from the photons incident on the cells to the.Â 5 ml of the lysate was used to estimate total protein (BCA Assay, Pierce Biotechnology Inc, Thermo-Scientific, Rockford, IL).Â 2. Karu TI (1998) The science of low power laser therapy. London, UK: Gordon and Breach Scientific Publications. 3. Karu T (1989) Photobiology of low-power laser effects. Health Phys 56: 691â€“704.