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Comparative study of the efficacy of pulsed electromagnetic field and low level laser therapy on mitogen-activated protein kinases

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ABSTRACT

Mitogen-Activated Protein Kinases (MAPKs) consist of three major signaling members: extracellular signal-regulated kinase (ERK), p38 and C-JUN N-terminal kinase (JNK). We investigated physiological effects of Pulsed Electromagnetic Field Therapy (PEMFT) and Low Level Laser Therapy (LLLT) on human body, adopting the expression level of mitogen-activated protein kinases as an indicator via assessment of the activation levels of three major families of MAPKS, ERK, p38 and JNK in the peripheral lymphocytes of patients before and after the therapies. Assessment for the expression levels of MAPKs families' were done, in the peripheral lymphocytes of patients recently have appendectomy, using flow cytometric analysis of multiple signaling pathways, pre and post LLLT and PEMFT application (twice daily for 6 successive days) on the appendectomy wound. There were non-significant differences in the expression levels of MAPKs families' pre- therapies application. But there were significant increase in the ERK expression levels post application of LLLT compared to its pre application ($p < 0.01$). Also, there was significant increase in the ERK, p38 and C-Jun N terminal expression level values post application of PEMFT compared to its pre application expression levels ($p < 0.01$ for each). The present study demonstrates that PEMFT has a powerful healing effect more than LLLT as it increase the activation of ERK, P38 and C-Jun-N Terminal while LLLT only increase the activation of ERK. LLLT has more potent pain decreasing effect than PEMFT as it does not activate P38 pathway like PEMFT.

1. Introduction

Lasers are often described by the kind of lasing medium they use-solid state, gas, exciter, dye or semiconductor. Lasers are also characterized by the duration of laser emission-continuous wave or pulsed laser (according to Oregon state university- faculty of Physics classification). The most common use of LLLT in the field of physical therapy is to promote healing process and decrease pain sensation after injury as LLLT has been suggested to enhance the activity of macrophages and fibroblast migration and proliferation and its subsequent increase in type I and III procollagen mRNA synthesis [1], it promote revascularization of wound [2], it also enhances bone healing, it also has visible stimulation of blood microcirculation in case of tissue heating on 0.8–1 °C or more [3], so it has potent effect in enhancing the healing process and in management of pain [4].

LLLT promote ATP synthesis [5] as cell absorb the radiated photons from laser and convert its energy to ATP which is a necessary source of energy for living cells in most of its functions. ATP is formed in cell mitochondria using glucose and oxygen in Krebs cycle to convert ADP to ATP. LLLT increases mitochondrial membrane potential and ATP synthesis in C2C21 myotubes with a peak response at 3–6 h [6]. This effect depends on chromophores which are light absorbing components in the cells and other cell components as cytochrome c, porphyrins and flavin also have a light absorbing capability [7]. Given explanation of this effect by the forming of singlet oxygen, reactive oxygen species (ROS), or nitric oxide. The enhanced ATP formation promote essential functions in the cell like cell homeostatic function, mitosis and proliferation by enhancing mRNA activity. The effect of laser depend on the time of application, intensity of the energy and exposure area, while, the depth of penetration depends on the wave length of the applied laser. High energy laser lead to cell damage [8,9]. While LLLT has a high effect on injured cells more than sound one that

Abbreviations: MAPKs, Mitogen-Activated Protein Kinases; ERK, Extracellular signal-Regulated Kinase; JNK, C-JUN N-terminal Kinase; PEMFT, Pulsed Electromagnetic Field Therapy; LLLT, Low Level Laser Therapy; DMEM, Dulbecco's Modified Eagle Medium

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Table 1
The ERK expression levels values (%), pre and post application of LLLT, PEMF on both groups.

ERK	Pre PEMF	Pre LLLT	Post PEMF	Post LLLT
Range	0.15–0.59	0.31–0.56	0.65–0.84	0.66–0.85
Mean ± SD	0.389 ± 0.120	0.455 ± 0.065	0.764 ± 0.062	0.768 ± 0.055
	Pre PEMF & Post PEMF	**Pre LLLT & Post LLLT**	**Post PEMF & Post LLLT**	
T. test	10.748	14.627	0.193	
P. value	0.001	0.001	0.853	

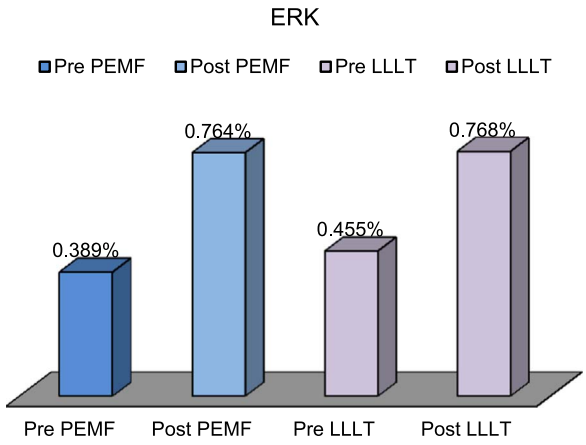

Fig. 1. The mean ERK expression levels (%) pre and post application of LLLT, PEMF on both groups.

give improve the hypothesis that laser promotes the normal cell function rather than changes it. Pulsed electromagnetic field therapy (PEMFT) creates micro currents in the body's tissues. These micro currents lead to certain biological and physiological responses depending on field parameters such as amplitude, frequency and wave form.

PEMFT is used worldwide and the most uses were to improve wound healing and fractures and pain modulation. Many theoretical hypotheses underlying this healing ability, one of them is due to increased blood circulation and microcirculation by significant arteriolar vasodilatation [10], anti-inflammatory effect [11], decrease edema and swelling [12], improve cell proliferation and differentiation potentials [13] and finally modulation of pain [14].

MAPKs are serine- threonine protein kinases that are activated by diverse stimuli ranging from cytokine, growth factors, neurotransmitters, hormones, cellular stress and cell adherence. MAPKs are expressed in all eukaryotic cells [15], they regulate diverse processes in the cell via transcriptional and non transcriptional control as they control the activity rhythm of large number of genes functionally related to each other's [16]. They consist of three major signaling members, the extracellular signal-regulated kinase (ERK), p38 and C-JUN N-terminal kinase (JNK). ERK regulates mitosis, proliferation, differentiation and survival of mammalian cells during development and neuronal plasticity in adult [17], while, p38 and JNK play essential roles in regulating inflammatory responses, neurodegeneration, cell death and pain hypersensation [18,19]. As conformational changes of any protein molecule affects its interaction with ligand and its biological partners at different level, so to understand the signal molecules interaction analysis, this could be explored by performing molecular docking with long term molecular dynamics simulations [20–22].

MAPKs are relates to various kinds of cellular stimulation and stress induced by heat, UV and chemicals, and they are important research targets in the field of biology and medicine. Application of electromagnetic field to therapy is also an interesting subject in which many things including its physical and biological mechanisms are still unclear. The current study provides the mechanisms of LLLT and PEMFT application in management of various human pathologies such as pain and wound healing via studying their effects on different signaling components of MAPKs pathway which is involved in various cellular regulatory functions including pain and wound healing process. Also the present study solves the conflicted studies regarding the effect of PEMFT on pain.

2. Material and methods

2.1. The study population

Fifty patients (30 male and 20 female) with age range (20–32) years old not suffering from any relevant diseases or take any medication that interfere with the application of treatment or measuring procedures and with no relevant history of smoking or addiction or bad habits or alcoholism who recently undergo appendectomy (3 days post-operative, as an example of injury) admitted at the department of surgery-Faculty of Medicine-Tanta University after obtaining approval of university hospital ethics committee and informed consent from the included patients. They were divided into 2 main groups: Group A (PEMFT) formed of 25 patients to whom the PEMFT has been applied. Group B (LLLT) formed of 25 patients to whom the LLLT has been applied.

2.2. PEMFT and LLLT applications

Portable magnetic therapy device (EASY Qs) was used for PEMFT application. This device has frequency from 5 to 100 Hz and intensity from 1 to 60 Gauss. Unidirectional quasi-rectangular waveform with strength 15 gauss and frequency less than 20 Hz was applied to the wound for 20–30 min each time. Pulsed wave diode laser (CEI 76-2/1999-1, Italy), was used for LLLT application, emitting wavelength of 905 nm with peak power 25 W and impulse duration 100nS. Linear application of LLLT on para-incisional line on both sides of the wound for 15–30 s for each point (total application period 20–30 min). Each of the two modalities was used twice daily for 6 successive days at the

Table 2
The P38 expression levels values (%), pre and post application of LLLT, PEMF on both groups.

P 38	Pre PEMF	Pre LLLT	Post PEMF	Post LLLT
Range	0.47–0.77	0.59–0.78	0.97–1.80	0.63–0.77
Mean+SD	0.669 ± 0.075	0.690 ± 0.061	1.269 ± 0.289	0.690 ± 0.039
	Pre PEMF & Post PEMF	**Pre LLLT & Post LLLT**	**Post PEMF & Post LLLT**	
T. test	7.783	0.001	7.686	
P. value	0.001	0.998	0.001	