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RESEARCH ARTICLE

BIOMECHANICS OF LOW LEVEL LASER THERAPY: A REVIEW.

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Abstract

Low Level Laser Therapy (LLLT) is a unique method of laser delivery that affects biological systems through non-thermal means. The photobiological response is the result of photo activated chemical or physical changes produced by the absorption of non-ionizing electromagnetic radiation by a viable cell. Mitochondrial enzymes being the natural chromophore are the likely initial acceptors of photon molecules and upon activation of a cascade of enzymatic activity contribute to the beneficial biological effects of LLLT. This review throws light on the molecular mechanisms upon exposure of biological tissue to low level lasers and its effects on gene expression of the cells.

Introduction:-

LASER - Light Amplification by Stimulated Emission of Radiation are equipments that generate electromagnetic radiation which are coherent, collimated and possess uniform wavelength. Laser radiation is produced by the active medium comprising of electrons in their excited state which slowly return to their stable ground energy state[(1)]. The photons thus emitted collide with other excited electrons which in turn causes further emission of photons. By travelling in synchronicity with its counterpart the photons cause a chain reaction, termed as population inversion and a light characteristic of the constituent active medium is produced.

Low Level Laser Therapy (LLLT) (photobiostimulation or photobiomodulation) is a unique method of laser delivery that affects biological systems through non-thermal means. LLLT comprises of exposing cells or tissue to Red or Near Infra-red light at energy densities that are meager compared to other forms of laser therapy for excision, ablation and coagulation of tissue.

The properties that define low level lasers are[(2)]:

1. Power output of lasers being 0.001- 0.1 Watts.
2. Wave length in the range of 300-10,600 nm.
3. Pulse rate from continuous wave to 5000 Hertz.
4. Intensity of the laser between 0.01-10 W/cm2
5. Dosage of laser in the range of 0.01 to 100 J/ cm^2.

The laser energy thus absorbed by the molecules and atoms of cells causes stimulation, without significant increase in tissue temperature. The Photobiological response is the result of photo activated chemical or physical changes produced by the absorption of non-ionizing electromagnetic radiation by a viable cell. LLLT works on the principle of Arndt-Schulz law which states that in a biological model, a weak and moderate stimuli acts as an excitant for physiological functions while contrastingly stronger stimuli retards function[(3)].

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According to Russian researchers; DNA during its replication cycle automatically emits light at 630 nm, close to the wavelength of the He Ne-laser (Helium Neon laser) light, it is hence postulated that laser may accelerate DNA replication via photonic stimulation[4]. The laser beam carries electromagnetic oscillations of a definite frequency which when reaches the tissues gradually "swing and excite" single cells. This is thought to eventually intensify the biochemical processes that ultimately regulate the performance of various vital organs[5].

Despite the availability of literature since mid-nineteenth century, there is still skeptism in acceptance of Low level laser therapy as a scientifically legitimate modality during therapy[6]. The lack of knowledge to principles of photobiology and the biological basis of LLLT are an important factor which contributes for its sparse application. This review aims at dissecting the molecular mechanism of low level laser therapy on oral tissues and their viable applications in the field of dentistry.

Theories of LLLT:-

The earliest theories on LLLT was based on the singlet oxygen hypothesis which states that cellular chromophores like porphyrins absorb the laser energy and in turn emit singlet oxygen. This singlet oxygen was theorized to stimulate the synthesis of RNA and DNA[7].

The Redox properties alteration hypothesis deals with excitation of chromatophores in the cytochrome C oxidase molecule which influences electron flow through the mitochondria and subsequently the redox status of the cell, thereby acting as a cell stimulant.

The latest most accepted hypothesis is the Nitrous Oxide hypothesis which suggests that electron flow stimulated by laser irradiation of cytrochrome C oxidase also reverse the partial down-regulation of the catalytic centre by nitrous oxide[8] .Laser absorption at the chromophore level increase the mitochondrial membrane potential and aids in the release of ATP and reactive oxygen species[9]. The increased energy generation and signal transduction affects the mitochondrial retrograde signaling under normal and pathological conditions. The cellular signaling is affected by the redox status of the cell, cellular reducing agents down-regulate the cellular signals while oxidative agents stimulate cell signaling by increasing communication in cells from mitochondria to the nucleus that influences many cellular activities.

Cytological Effects Of Low Level Laser Therapy:-

Effect on Mitochondria:-

The mechanism of action of LLLT is through mitochondrial protein Cytochromase c oxidase (COX) which is the terminal enzyme of the electron transport chain and plays a vital role in regulating cell metabolism. Cox is the primary chromophore in mammalian cells subjected to near infra red radiation as it has the same absorption spectra to elicit a biological response[10,11]. The acceptance of the photon by Cox results in rapid electron transfer reactions leading to an electronically excited state. This ultimately results in increased ATP production and upregulates sodium-potasium-ATP pump (Na+/K+/ATPase), calcium (Ca^{2+})pumps. ATP also indirectly influences the regulation of cyclic AMP (cAMP). Both cAMP and Ca^{2}+ regulates numerous cellular functions and are important second messengers of the cell [12,13].

Nitric Oxide and LLLT:-

Cox contains a heme binuclear centre and binuclear copper centre (CuA) through which it can transfer electrons from water to oxygen to form oxygen free radicals. This reaction is reversibly inhibited by nitric oxide (NO) by competitively binding to the CuA centre[14]. It is theorized that irradiation by laser light can lead to dissociation of NO from its binding site and increases the respiration rate of the cell. This also protects the cell from NO induced cell death[15].

Redox activity of the cell:-

The irradiation of cell with laser increases the production of reactive oxygen species (ROS) within the cell and there is a shift towards greater oxidation within the cells. Increased ROS stimulated a number of cellular signaling channels and events such as nucleic acid synthesis, enzyme activation and protein synthesis[16,17]. LLLT also affects cell cycle progression by controlling the gene expression. A study by Zhang et al on human fibroblasts subjected to irradiation by low level light showed that LLLT can affect gene expression in these cells[18]. Irradiation of the cells stimulates expression of genes related to cell proliferation and regulates cell growth. It also influences genes related to cell migration and remodeling, DNA synthesis and repair and cell metabolism.

Prevention of apoptosis:-

One of the major effects of LLLT is reversal of inhibition of the Cox by NO or any other inhibitory molecules, and consequently lowers the likelihood of apoptosis in many conditions.Studies by Eells et al and Wong-Riley et al have shown that Near Infra-Red (NIR) irradiation could reduce the rate of apoptosis by 50% in cultured neurons[19,20]. The release of Cox from mitochondria into the cytoplasm activates the apoptotic pathway by the upregulation of caspase-3 and hence acts as potent apoptotic signal. LLLT maintains Cox activity by preventing its release into the cytoplasm by promoting Bcl-2 expression and inhibits Bax expression [21].

Stimulation of angiogenesis:-

Various animal models which studied wound healing after mechanically inducing wounds or burns on animals report that, exposed animals were capable of forming increased granulation tissue and enhanced epithelialisation and phagocytosis as opposed to control groups[22,23,24]. These cumulative effects of LLLT on wound healing were attributed to its effects on angiogenesis or stimulation of growth of new blood vessels. The mechanism by which LLLT stimulates angiogenesis was brought to light by the work of Hagen et al who studied the effect of NO in stimulation the hypoxia factor (HIF1α) [25]. Inhibiton of mitochondrial respiration is an important consequence of hypoxia and its subsequent reversal by exposure to LLLT stabilizes HIF1 α which is a potent stimulator of vascular endothelial growth factor (VEGF)[26]. VEGF is the most potent stimulator of proliferation and migration of endothelial cells and new blood vessel formation.

Gene expression and LLLT:-

Cell culture studies involving human fibroblasts reveal that LLLT can have profound effect on the expression of various genes and their functional products (Table 1)[27]. LLLT aids in cell proliferation by modulating the respective genes and also downregulates cell apoptosis.

Table 1:- The influence of LLLT on gene level expression and its cellular effects

Upregulation of Gene		**Downregulation of Gene**	
Gene	**Effect**	**Gene**	**Effect**
• Mitogen activated protein kinase 11 • Break point cluster region • Serum response factor	• Proliferation	• Heat Shock 70kD protein • Cullin 1	Prevent proliferation Apoptosis
• NADH dehydrogenase 1β • ATP synthase • Electron transfer flavoprotein	• Energy metabolism and respiratory chain		

Conclusion:-

Thus the molecular mechanism of LLLT suggest that the photons absorbed by the chromophore in the mitochondria stimulate production of ATP and down regulation of reactive oxygen species; thereby activating favourable factors which induce suitable gene expression and mediate the valuable effects of LLLT at the cellular level. Further research in the molecular mechanisms of LLLT will lead to greater acceptance of the treatment modality and evolve as a definitive adjuvant in mainstream medicine and dentistry.

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