Review Article

Biological Effects of Low Level Laser Therapy

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Abstract:

The use of low level laser to reduce pain, inflammation and edema, to promote wound, deeper tissues and nerves healing, and to prevent tissue damage has been known for almost forty years since the invention of lasers. This review will cover some of the proposed cellular mechanisms responsible for the effect of visible light on mammalian cells, including cytochrome c oxidase (with absorption peaks in the Near Infrared (NIR)). Mitochondria are thought to be a likely site for the initial effects of light, leading to increased ATP production, modulation of reactive oxygen species, and induction of transcription factors. These effects in turn lead to increased cell proliferation and migration (particularly by fibroblasts).

Keywords: low level laser therapy; cytochrome c oxidase; reactive oxygen species; cell proliferation; cell migration

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Introduction

Lasers (Light amplification by stimulated emission of radiation) are devices that typically generate electromagnetic radiation which are relatively uniform in wavelength, phase, and polarization, originally described by Theodore Maiman in 1960 in the form of a ruby laser [1].

Laser is described as a source of light or radiation energy [2]. Low Level Laser (LLL) is a special type of laser that effects on biologic systems through non-thermal means [3]. This area of investigation started with the work of Mester et al in 1967. They reported non-thermal effects of lasers on mouse hair growth [4].

According to Posten et al, properties of low level lasers are:

a) Power output of lasers being 0.001- 0.1 Watts.
b) Wave length in the range of 300-10,600 nm.
c) Pulse rate from 0, meaning continuous to 5000 Hertz (cycles per second).
d) Intensity of 0.01-10 W/cm^2 and dose of 0.01 to 100 J/ cm^2 [5].

Most common methods of administration of LLL radiation include lasers such as ruby (694 nm), Ar (488 and 514 nm), He-Ne (632.8 nm), Krypton (521, 530, 568, and 647 nm), Ga-Al-As (805 or 650 nm), and Ga-As (904 nm) [3].

Low Level Laser therapy (LLLT) is the application of light to a biologic system to promote tissue regeneration, reduce inflammation and relieve pain. Unlike other medical laser procedures, LLLT does not have an ablative or thermal mechanism, but rather a photochemical effect which means the light is absorbed and cause a chemical change [6]. The reason why the technique is termed low level is that the optimum levels of energy density delivered are low and it is not comparable to other forms of laser therapy as practiced for ablation, cutting, and thermal tissue coagulation [7].

The first law of photobiology explains that for a low power visible light to have any effect on a living biological system, the photons must be absorbed by electronic absorption bands belonging to some molecular photo-acceptors, which are called chromophores [8]. The effective tissue penetration of light at 650 nm to 1200 nm is maximized. The absorption and scattering of

light in tissue are both much higher in the blue region of the spectrum than the red, because the main tissue chromophores (hemoglobin and melanin) have high absorption bands at shorter wavelengths and tissue scattering of light is higher at shorter wavelengths. Water strongly absorbs infrared light at wavelengths greater than 1100 nm. Therefore, the use of LLLT in animals and patients almost exclusively utilizes red and near-infrared light (600-1100 nm) [9].

Mitochondrial Respiration and ATP

Current research about the mechanism of LLLT involves mitochondria [6]. Cytochrome c oxidase (Cox) is a multicomponent membrane protein that contains a binuclear copper center (CuA) along with a heme binuclear center (a3-CuB), both of which facilitate the transfer of electrons from water soluble cytochrome c oxidase to oxygen. It is a terminal enzyme of the electron transport chain and plays a vital role in the bioenergetics of a cell [11]. It was proposed that Cox is the primary photoacceptor for the red-NIR range in mammalian cells because absorption spectra obtained for Cox in different oxidation states was found to be very similar to the action spectra for biological responses to light [10].

The absorption of photons by Cox leads to electronically excited states, and consequently can lead to quickening of electron transfer reactions [12]. More electron transport necessarily causes increased production of ATP [13].

The light induced increase in ATP synthesis and increased proton gradient lead to an increasing activity of the Na+/H+ and Ca2+/Na+ antiporters, and of all the ATP driven carriers for ions, such as Na+/K+ ATPase and Ca2+ pumps. ATP is the substrate for adenylcyclase, and therefore the ATP level controls the level of cAMP. Both Ca2+and cAMP are very important second messengers. Ca2+ regulates almost every process in the human body (muscle contraction, blood coagulation, signal transfer in nerves, gene expression, etc…) [7]. Therefore the photoactivation of terminal enzymes, like Cox, plays a vital role in the activation of the diverse biological cascade observed subsequently to laser irradiation.

Nitric Oxide and LLLT

The activity of cytochrome c oxidase is inhibited by nitric oxide (NO) [14, 15]. This inhibition can be explained by a direct competition between NO and O2 for the reduced binuclear center CuB/a3 of cytochrome c oxidase, and is reversible [16]. It was proposed that laser irradiation could reverse this inhibition by photodissociating NO from its binding sites [17, 18]. Because this coordinate binding is much weaker than a covalent bond, this dissociation is possible by LLL. The dissociation of NO from Cox increases the respiration rate [18]. Light can indeed reverse the inhibition caused by NO binding to cytochrome oxidase, both in isolated mitochondria and in whole cells [19]. LLL can also protect cells against NO-induced cell death [7].

Reactive Oxygen Species (ROS) and Gene Transcription

LLLT was reported to produce a shift in overall cell redox potential in the direction of greater oxidation [20] and increased ROS generation and cell redox activity have been reported [21,22,23,24,25]. It has been proposed that the redox state of a cell regulates cellular signaling pathways that control gene expression. Modulation of the cellular redox state can activate or inhibit signaling pathways [11]. Several regulation pathways are mediated through the cellular redox state. Changes in redox state induce the activation of numerous intracellular signaling pathways, such as nucleic acid synthesis, protein synthesis, enzyme activation and cell cycle progression [26].

These cytosolic responses may induce transcriptional changes. Several transcription factors have been recognized to regulate by changes in cellular redox state. Among them redox factor-1 (Ref-1)-dependent activator protein-1 (AP-1) (Fos and Jun), nuclear factor B (NF-B), p53, activating transcription factor/cAMP-response element-binding protein (ATF/ CREB), hypoxia-inducible factor (HIF)-1 and HIF-like factor are the most important factors [7].

Based on the ability of LLLT to modulate cellular metabolism and alter the transcription factors responsible for gene expression, it has been found to alter gene expression [27].

LLL & Gene Expression

The gene expression profiles of human fibroblasts irradiated by low-intensity red light show that the irradiation can affect the expression of many genes that belong to different function categories [36]. Irradiation of LLL stimulates cell growth directly through regulation of the expression of genes related to cell proliferation and indirectly through regulation of the expression of genes related to cell migration and remodeling, DNA synthesis and repair, ion channel and membrane potential, and cell

metabolism. Irradiation by red light also enhances cell proliferation by suppression of cell apoptosis [36]. Table 1 shows some of these genes.

The Usage of LLLT

The temporomandibular disorders (TMDs) have been identified as the most important cause of pain in the facial region. Low Level laser therapy (LLLT) has demonstrated to have analgesic, anti-inflammatory and biostimulating effects. The LLLT is a noninvasive, quick and safe, non-pharmaceutical intervention that may be beneficial for patients with TMDs [37].

Another study proposed a novel combination of neural regeneration techniques for the repair of damaged peripheral nerves. A biodegradable nerve conduit containing genipin-cross-linked gelatin was annexed using beta-tricalcium phosphate (TCP) ceramic particles (genipin-gelatin-TCP, GGT) to bridge the transection of a 15mm sciatic nerve in rats. Electrophysiological measurements (peak amplitude and area) illustrated by compound muscle action potential (CMAP) curves demonstrated that laser stimulation significantly improved nerve function and reduced muscular atrophy. Histomorphometric assessments revealed that laser stimulation accelerated nerve regeneration over a larger area of neural tissue, resulting in axons of greater diameter and myelin sheaths of greater thickness than that observed in rats treated with nerve conduits alone [38].

Summary

The molecular and cellular mechanisms of LLLT suggest that photons are absorbed by the mitochondria. They stimulate more ATP production and low levels of ROS, which then activates transcription factors, such as NF-κB, to induce many gene transcript products responsible for the beneficial effects of LLLT. ROS are well known to stimulate cellular proliferation of low levels, but inhibit proliferation and kill cells at high levels. Nitric oxide is also involved in LLLT, and may be photo-released from its binding sites in the respiratory

Table 1. The effects of LLL on gene expression.

Name of genes	Role	Change	Mechanism
Mitogen-activated protein kinase 11 (MAPK11)	Proliferation	Up-regulation	Isoform of the p38 MAPK, which signaling pathway is involved in fibroblast growth factor2 induced proliferation[28]
Breakpoint cluster region (BCR)	Proliferation	upregulation	A GTPase-activating protein for Ras-related C3 botulinum toxin substrate 1 (RAC1) and Cell division control protein 42(CDC42) that promotes the exchange of RAC- or CDC42- bound GDP by GTP. Active RAC1 and CDC42 can suppress p21, leading to the upregulation of BCR gene, which can enhance cell growth[29]
Platelet derived growth factor C (PDGF-C)	Proliferation	upregulation	A member of the PDGF/vascular endothelial growth factor family and its upregulation can induce mitogenic activity on several mesenchymal cell types[30]
Serum response factor	Proliferation	upregulation	It contributes to mitogen-stimulated transcriptional induction of many immediate-early genes during the G0-G1 cell cycle transition and is also essential for cell cycle progression[31]
Cullin 1	Prevent Proliferation	downregulation	The downregulated gene cullin 1 is an inhibitory regulator of the cell cycle. Cullin 1 is required for developmentally programmed transitions from the G1 phase to the G0 phase of the cell cycle or the apoptotic pathway, the mutation of which leads to the acceleration of G1 to S phase progression[32]
Heat shock 70kD protein 1A Caspase 6 Stress induced-phosphoprotein 1	Apoptosis	downregulation	Apoptpsis
NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 2	Energy metabolism and respiratory chain	upregulation	It is one of the peptides of mitochondria respiratory complex I that transfer electrons from NADH to the respiratory chain[33]
ATP synthase, H þ transporting, mitochondrial F0 complex, subunitd	Energy metabolism and respiratory chain	upregulation	ATP5H belongs to the respiratory complex V (F1F0 -ATPase assembly), which catalyzes ATP synthesis[34]
Electron-transfer-flavoprotein, beta polypeptide	Energy metabolism and respiratory chain	upregulation	Electron-transfer-flavoprotein b polypeptide (ETFB) is a subunit of ETF that serves as a specific electron acceptor for several dehydrogenases including acyl-CoA dehydrogenases that function in fatty acid b oxidation[35]

chain and elsewhere. It is possible that NO release in low amounts by low dose light may be beneficial.

Further advances in the mechanistic understanding of LLLT will continue to be made in the near future. These advances will lead to greater acceptance of LLLT in main- stream medicine and may lead to LLLT being used for serious diseases such as stroke, heart attack and degenerative brain diseases.

References

1. Maiman TH. Stimulated optical radiation in Ruby. Nature 1960;187:493-94.
2. Verma SK, Maheshwari S, Singh RK, Chaudhari PK. Laser in dentistry: An innovative tool in modern dental practice. Natl J Maxillofac Surg 2012;3(2):124-32.
3. Lin F, Josephs SF, Alexandrescu DT, Ramos F, Bogin V, Gammill V, et al. Lasers, stem cells, and COPD. J Trans Med 2010; 8:16.
4. Mester E, Szende B, Tora JG. Effect of laser on hair growth of mice. Kiserl Orvostud 1967; 19:628-31.
5. Posten W, Wrone DA, Dover JS, Arndt KA, Silapunt S, Alam M. Low-level laser therapy for wound healing: mechanism and efficacy. Dermatol Surg 2005; 31(3):334-40.
6. Huang YY, Chenet AC, Carroll JD, Hamblin RM. Biphasic dose response in low level light therapy. Dose Response 2009; 7(4):358-83.
7. Hamblin MR. Mechanisms of low level light therapy. Proc. of SPIE 2009; 6140: 614001-1.
8. Sutherland JC. Biological effects of polychromatic light. Photochem Photobiol 2002; 76(2):164-70.
9. Karu TI, Afanas'eva NI. [Cytochrome c oxidase as the primary photoacceptor upon laser exposure of cultured cells to visible and near IR-range light]. DoklAkadNauk 1995; 342(5):693-5. Russian.
10. Karu TI, Kolyakov SF. Exact action spectra for cellular responses relevant to phototherapy. Photomed Laser Surg 2005; 23(4):355-61.
11. Srinivasan S, Avadhani NG. Cytochrome c oxidase dysfunction in oxidative stress. Free Radic Biol Med 2012; 53(6): 1252-63.
12. Yu W, Naim JO, McGowan M, Ippolito K,Lanzafame RJ. Photomodulation of oxidative metabolism and electron chain enzymes in rat liver mitochondria, Photochem Photobiol 1997;66(6): 866-71.
13. Passarella S. He-Ne laser irradiation of isolated mitochondria. J Photochem Photobiol 1989; 3(4): 642-3.
14. Beltran B, Mathur A, Duchen MR, Erusalimsky JD, Moncada S. The effect of nitric oxide on cell respiration: A key to understanding its role in cell survival or death, Proc Natl Acad Sci U S A 2000; 97(26):14602-7.
15. Brown GC. Regulation of mitochondrial respiration by nitric oxide inhibition of cytochrome c oxidase, Biochim Biophys Acta 2001; 1504(1): 46-57.
16. Antunes F, Boveris A, Cadenas E. On the mechanism and biology of cytochrome oxidase inhibition by nitric oxide, Proc Natl Acad Sci U S A 2004; 101(48): 16774-9.
17. Lane N. Cell biology: power games. Nature 2006;443(7114): 901-3.
18. Karu TI, PyatibratLV, Afanasyeva NI. Cellular effects of low power laser therapy can be mediated by nitric oxide. Lasers Surg Med 2005;36(4): 307-14.
19. Borutaite V, Budriunaite A, Brown GC. Reversal of nitric oxide-, peroxynitrite- and S-nitrosothiol-induced inhibition of mitochondrial respiration or complex I activity by light and thiols. Biochim Biophys Acta 2000; 1459(2-3): 405-12.
20. Karu T. Primary and secondary mechanisms of action of visible to near-IR radiation on cells. J Photochem Photobiol 1999; 49(1):1-17.
21. Alexandratou E, Yova D, Handris P, Kletsas D, Loukas S. Human fibroblast alterations induced by low power laser irradiation at the single cell level using confocal microscopy. Photochem Photobiol Sci 2002; 1(8):547-52.
22. Chen AC-H, Arany PR, Huang YY, Tomkinson EM, Saleem T, Yull FE, et al. Low level laser therapy activates NF-κB via generation of reactive oxygen species in mouse embryonic fibroblasts. Proc SPIE in press 2012.
23. Grossman N, Schneid N, Reuveni H, Halevy S, Lubart R. 780 nm low power diode laser irradiation stimulates proliferation of keratinocyte cultures: involvement of reactive oxygen species. Lasers Surg Med 1998; 22(4):212-8.
24. Lavi R, Shainberg A, Friedmann H, Shneyvays V, Rickover O, EichlerM, et al. Low energy visible light induces reactive oxygen species generation and stimulates an increase of intracellular calcium concentration in cardiac cells. J Biol Chem 2003; 278(42):40917-22.
25. Zhang J, Xing D, Gao X. Low-power laser irradiation activates Src tyrosine kinase through reactive oxygen species-mediated signaling pathway. J Cell Physiol 2008; 217(2):518-28.
26. Liu H, Colavitti R, Rovira II, Finkel T. Redox- dependent transcriptional regulation. Circ Res 2005; 97(10): 967-74.
27. Byrnes KR, Wu X, Waynant RW, Ilev IK, Anders JJ. Low power laser irradiation alters gene expression of olfactory ensheathing cells in vitr. Lasers Surg Med 2005;37(2):161-171.
28. Maher P. Phorbol esters inhibit fibroblast growth factor-2-stimulated fibroblast proliferation by a p38 MAP kinase dependent pathway. Oncogene 2002; 21(13):1978-88.
29. Bao W, Thullberg M, Zhang H, Onischenko A, Stromblad S. Cell attachment to the extracellular matrix induces proteasomal degradation of p21 (CIP1) via Cdc42/ Rac1 signaling. Mol Cell Biol 2002; 22:4587-97.
30. Gilbertson DG, Du¡ ME, West JW, Kelly JD, Sheppard PO, Hofstrand PD, et al. Platelet-derived growth factor C (PDGF- C), a novel growth factor that binds to PDGF a and b receptor. J Biol Chem 2001; 276(29):27406-14.
31. Schratt G, Weinhold B, Lundberg AS,Schuck S, Berger J, Schwarz H, et al. Serum response factor is required for

immediate-early gene activation yet is dispensable for proliferation of embryonic stem cells. Mol Cell Biol 2001; 21(8): 2933-43.

32. Kipreos ET, Lander LE, Wing JP, He WW, Hedgecock EM. Cul-1 is required for cell cycle exit in C. elegans and identifies a novel gene family. Cell 1996; 85(6):829-39.
33. Weiss H, Friedrich T, Hofhaus G, Preis D. The respiratory-chain NADH dehydrogenase (complex I) of mitochondria. Eur J Biochem 1991; 197(3):563-76.
34. Walker JE, Collinson IR. The role of the stalk in the coupling mechanism of F1F0- ATPases. FEBS Lett 1994; 346(1): 39-43.
35. Thorpe C, Kim JJ. Structure and mechanism of action of the acyl-CoA dehydro- genases. FASEB J 1995; 9(9):718-25.
36. Zhang Y, Song S, Fong CC, Tsang CH, Yang Z,Yang M. cDNA Microarray Analysis of Gene Expression Profiles in Human Fibroblast Cells Irradiated with Red Light. *Journal of Investigative* Dermatology 2003; 120: 849–57.
37. Herranz-Aparicio J, Vázquez-Delgado E, Arnabat-Domínguez J, España-Tost A, Gay-Escoda C. The use of low level laser therapy in the treatment of temporomandibular joint disorders. Oral Medicine and Pathology.Med Oral Patol Oral Cir Bucal 2013;18(4): e603–e12.
38. Shen CC, Yang YC, Huang TB, Chan SC, Liu BS. Low-Level Laser-Accelerated Peripheral Nerve Regeneration within a Reinforced Nerve Conduit across a Large Gap of the Transected Sciatic Nerve in Rats. Evid Based Complement Alternat Med 2013; Article ID 175629, 12 pages.