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Role of Reactive Oxygen Species in Low Level Light Therapy.

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ABSTRACT

This review will focus on the role of reactive oxygen species in the cellular and tissue effects of low level light therapy (LLLT). Coincidentally with the increase in electron transport and in ATP, there has also been observed by intracellular fluorescent probes and electron spin resonance an increase in intracellular reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, singlet oxygen and hydroxyl radical. ROS scavengers, antioxidants and ROS quenchers block many LLLT processes. It has been proposed that light between 400-500-nm may produce ROS by a photosensitization process involving flavins, while longer wavelengths may directly produce ROS from the mitochondria. Several redox-sensitive transcription factors are known such as NF-kB and AP1, that are able to initiate transcription of genes involved in protective responses to oxidative stress. It may be the case that LLLT can be pro-oxidant in the short-term, but anti-oxidant in the long-term.

Keywords: biostimulation, low level laser therapy, mitochondria, reactive oxygen species, redox-sensitive signal transduction, transcription factor,

1 INTRODUCTION

Low-level light (or laser) therapy (LLLT) has been applied in various areas of medicine for over forty years. It was discovered by Endre Mester in Semmelweis University, Budapest, Hungary in 1967 a few years after the first working laser was invented. Mester wanted to test if laser radiation might cause cancer in mice [1]. He shaved the dorsal hair, divided them into two groups and gave a laser treatment with a low powered ruby laser (694-nm) to one group. They did not get cancer and to his surprise the hair on the treated group grew back more quickly than the untreated group. This was the first demonstration of "laser biostimulation". Since then the range of applications of LLLT have broadened to include wound healing, stimulation of cartilage and bone repair, prevention of tissue death, pain relief, reduction of inflammation and swelling and many more indications of both acute and chronic injuries and degenerative diseases. It has also been termed "cold laser", "soft laser", "biostimulation" or "photobiomodulation".

Despite forty-plus years of accumulating experience of the clinical efficacy of LLLT, the basic molecular and cellular mechanisms that underlie the biostimulation effect remain uncertain. The aim of this review chapter is to explore the role of the generation of reactive oxygen species (ROS) during LLT and to cover some of the signaling pathways that can be activated as a response to these ROS.

2 MOLECULAR MECHANISMS OF LLLT

2.1. Chromophores

The first law of photobiology states that for 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 chromophore or photoacceptor [2]. One approach to finding the identity of this chromophore is to carry out action spectra. This is a graph representing biological photoresponse as a function of wavelength, wave number, frequency, or photon energy and should resemble the absorption spectrum of the photoacceptor molecule. The fact that a

structured action spectrum can be constructed supports the hypothesis of the existence of cellular photoacceptors and signaling pathways stimulated by light.

Wavelengths that have been reported to have stimulating effects in biological systems include blue (400-500-nm), green (510-550-nm) yellow (560-590-nm), red (600-700-nm) and near-infrared (710-1100-nm). There have even been some reports of broadband white light producing biological stimulation. Different chromophores have been proposed to be operating as photoacceptors depending on the wavelengths chosen and the precise biological system under investigation. Blue and green (and white light) have been proposed to be absorbed by flavins and flavoproteins, yellow and red light have been proposed to be absorbed by porphyrins, and red and near infrared light have been proposed to be absorbed by cytochrome c oxidase.

Of the three groups of chromophores described above, cytochrome c oxidase has attracted the most attention. This protein is unit 4 of the cellular respiratory chain located in the inner mitochondrial membrane. The respiratory chain consists of five linked complexes of integral membrane proteins: NADH dehydrogenase (Complex I), succinate dehydrogenase (Complex II), cytochrome c reductase (Complex III), cytochrome c oxidase (Complex IV), and ATP synthase (Complex V).

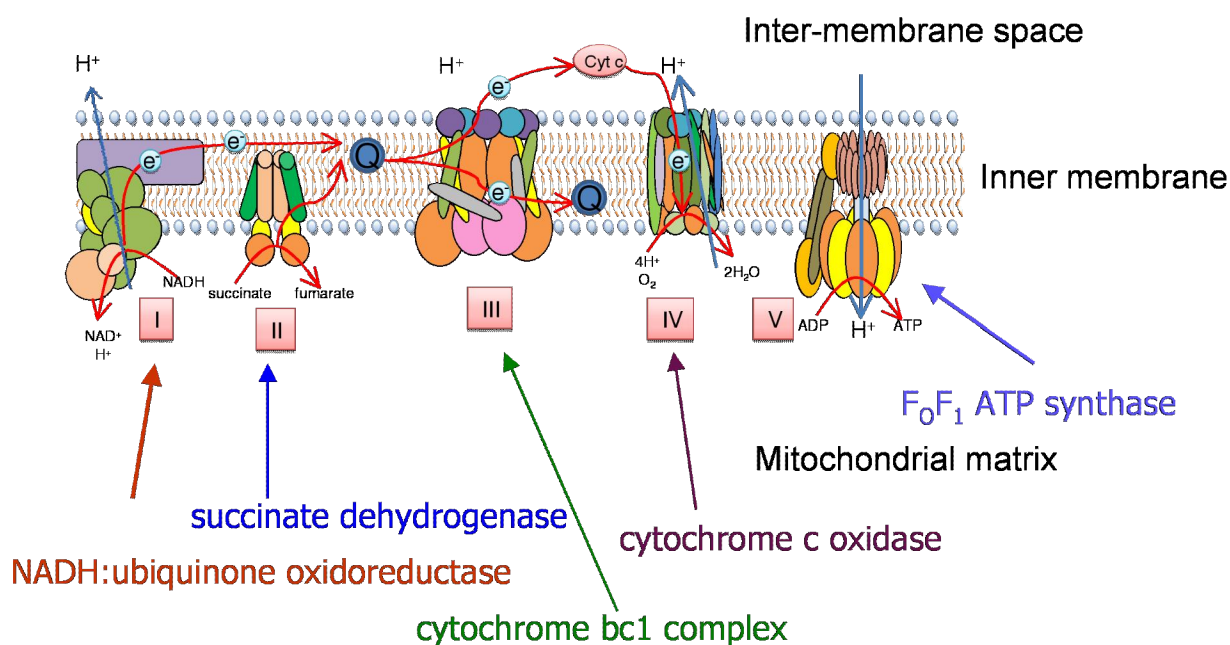


Figure 3. Structure of the mitochondrial respiratory chain

The respiratory chain accomplishes the stepwise transfer of electrons from NADH and FADH₂ (produced in the citric acid or Krebs cycle) to oxygen molecules to form (with the aid of protons) water molecules harnessing the energy released by this transfer to the pumping of protons (H⁺) from the matrix to the intermembrane space. The gradient of protons formed across the inner membrane by this process of active transport forms a miniature battery. The protons can flow back down this gradient, reentering the matrix, only through another complex of integral proteins in the inner membrane, the ATP synthase complex.

Absorption spectra obtained for cytochrome c oxidase in different oxidation states were recorded and found to be very similar to the action spectra for biological responses to light. Cytochrome C oxidase contains two iron centers, heme *a* and heme *a*₃ and two copper centers, Cu_A and Cu_B [3]. Fully oxidized cytochrome c oxidase has both iron atoms in the Fe(III) oxidation state and both copper atoms in the Cu(II) oxidation state, while fully

reduced cytochrome c oxidase has the iron in Fe(II) and copper in Cu(I) oxidation states. There are many intermediate mixed-valence forms of the enzyme and other coordinate ligands such as CO, CN, and formate can be involved. All the many individual oxidation states of the enzyme have different absorption spectra [4], thus probably accounting for slight differences in action spectra of LLLT that have been reported. A recent paper from Karu's group [5] gave the following wavelength ranges for four peaks in the LLLT action spectrum: 1) 613.5 - 623.5 nm, 2) 667.5 - 683.7 nm, 3) 750.7 - 772.3 nm, 4) 812.5 - 846.0 nm.

2.2 Tissue optics.

An important consideration involves the optical properties of tissue. Both the absorption and scattering of light in tissue are wavelength dependent (both much higher in the blue region of the spectrum than the red) and the principle tissue chromophore (hemoglobin) has high absorption bands at wavelengths shorter than 600-nm. Water begins to absorb significantly at wavelengths greater than 1150-nm. For these reasons there is a so-called "optical window" in tissue covering the red and near-infrared wavelengths, where the effective tissue penetration of light is maximized. Therefore although blue, green and yellow light may have significant effects on cells growing in optically transparent culture medium, the use of LLLT in animals and patients almost exclusively involves red and near-infrared light (600-1100-nm).

2.3 Release of nitric oxide from cytochrome c oxidase – a hypothesis

Red and near-IR light in cells is primarily absorbed by cytochrome c oxidase (unit four in the mitochondrial respiratory chain). Nitric oxide produced in the mitochondria can inhibit respiration by binding to cytochrome c oxidase and competitively displacing oxygen, especially in stressed or hypoxic cells. If light absorption displaced the nitric oxide and thus allowed the cytochrome c oxidase to recover and cellular respiration to resume, this would explain many of the observations made in LLLT. Increases in ATP are one of the most often observed changes after LLLT is carried out in vitro and increased cytochrome c oxidase activity would explain raised ATP levels. Hypoxic, stressed or damaged cells or tissues are likely to respond more to LLLT than normal cells and tissues, and their cytochrome c oxidase is more likely to be operating at sub-optimal level due to NO inhibition. LLLT effects can keep working for some time (hours or days) post-illumination because the displaced nitric oxide cannot easily return to inhibit cytochrome c oxidase. Increased NO concentrations can sometimes be measured in cell culture or in animals after LLLT due to its release from the mitochondria and cytochrome c oxidase. The release of NO from the mitochondria could explain the transient increases in blood flow measured in skin microcirculation after LLLT. Release of NO into the tissue could also explain the effect of LLLT in increasing lymphatic drainage in conditions like lymphedema and reducing swelling in trauma. The removal of the inhibiting NO from cytochrome c oxidase will lead to a sharp drop in pO_2 in the cell as respiration resumes and if the cells have relatively low pO_2 levels to start with, this drop will lead to hypoxic signaling through stabilization of HIF-1 α . Hypoxic signaling is one of the main factors leading to VEGF synthesis and its consequent increase in angiogenesis as has been observed after LLLT for wound healing.

2.4 Laser speckle effects in mitochondria

An alternative explanation for LLLT effects on cells and mitochondria does not rely on specific photon absorption by defined wavelength bands in chromophores leading to photochemistry, but more relies on non-specific photon absorption leading to photothermal effects. Since the total energy delivered is small the resulting rise in temperature would be insignificant if the energy was evenly distributed over the whole cell. However in the case of coherent laser light the energy is not evenly distributed, but forms a speckle pattern. When a surface is illuminated by a light wave, according to diffraction theory, each point on an illuminated surface acts as a source of secondary spherical waves. Laser speckle is formed by interference (either constructive or destructive) of waves that have been scattered from each point on the illuminated surface. If the surface is rough enough to create path length differences exceeding one wavelength, giving rise to phase changes greater than 2π , the amplitude, and hence the intensity, of the resultant light varies randomly. If light of low coherence (i.e. made up of many wavelengths) is used, a speckle pattern will not normally be observed, because the speckle patterns

produced by individual wavelengths have different dimensions and will normally average one another out. The "size" of the speckles is a function of the wavelength of the light, the size of the laser beam that illuminates the first surface, and the distance between this surface and the surface where the speckle pattern is formed. In tissue the diameter of the speckles is of the order of the wavelength of light i.e. about 1 micron and this dimension is comparable to the size of the mitochondria. The hypothesis is that the laser speckles produce micro-thermal gradients due to inhomogeneous energy absorption that can stimulate or otherwise alter the metabolism within mitochondria [6].

This hypothesis would explain reports that some LLLT effects in cells and tissues are more pronounced when coherent laser light is used than comparable non-coherent light from LED or filtered lamp sources that is of similar wavelength range although not monochromatic [7].

2.5 Mitochondrial response to LLLT

Several groups have tested the effect of LLLT on preparations of isolated mitochondria. HeNe laser illumination of mitochondria isolated from rat liver led to increased proton electrochemical potential and ATP synthesis [8]. Increased RNA and protein synthesis was demonstrated after 5 J/cm² [9]. Pastore et al [10] found increased activity of cytochrome c oxidase and an increase in polarographically measured oxygen uptake after 2 J/cm² of HeNe. A major stimulation in the proton pumping activity was found in illuminated mitochondria. Yu et al [11] used 660 nm laser at a power density of 10 mW/cm² and showed increased oxygen consumption (0.6 J/cm² and 1.2 J/cm²), increased phosphate potential, and energy charge (1.8 J/cm² and 2.4 J/cm²) and enhanced activities of NADH: ubiquinone oxidoreductase, ubiquinol: ferricytochrome C oxidoreductase and ferrocycytochrome C: oxygen oxidoreductase (between 0.6 J/cm², and 4.8 J/cm²).

3 PRODUCTION OF REACTIVE OXYGEN SPECIES BY LLLT

There have been multiple reports from several laboratories that LLLT carried out in vitro leads to generation of various kinds of ROS within the illuminated cells and in the medium surrounding them. Lubart and her laboratory have published several papers on generation of ROS after LLLT [12, 13]. They reported that white light as well as non-coherent blue green and red/NIR light could all produce ROS [14]. ROS have mainly been detected by electron spin resonance (ESR) techniques. ESR involves the addition of a spin-trapping probe such as TEMPO, DMPO and DEPMPO that will produce a characteristic and measurable signal in the presence of particular ROS. Superoxide anion, hydroxyl radical and singlet oxygen can all be individually detected by using this technique. Cardiac and sperm cells were examined during illumination by a filtered halogen lamp [15]. Oxyradicals were created solely by the 400-500 nm range of visible light. The endogenous photosensitizer was found predominantly in the cytosol and was smaller than 12 kDa. Flavin mononucleotide produced the same signal at concentrations consistent with reported intracellular free flavin concentrations. Increases in cytosolic calcium were also seen that could be reduced by verapamil, a voltage-dependent calcium channel inhibitor [16]. Extracellular hydrogen peroxide was also detected and addition of extracellular catalase reduced the increase in calcium.

A 780-nm diode laser stimulated proliferation of cultured normal human keratinocytes [17]. A single dose of 0.45-0.95 J/cm² of irradiation increased incorporation of 3H-thymidine during 6-24 hr following irradiation; and increased the percentage of dividing cells and number of cells, 24 hr and 48 hr following irradiation, respectively. Added enzymatic antioxidants, superoxide dismutase or catalase, scavenging superoxide anions and H₂O₂, suppressed this enhanced proliferation. Added scavengers (alpha-tocopherol acetate, scavenging lipid peroxidation, or sodium azide, histidine, mannitol, scavenging singlet oxygen, superoxide anions, and hydroxyl radicals, respectively), or N-acetyl cysteine, the thiol-reducing agent, suppressed the response, but to different extents.

Callaghan et al [18] used a diode laser (660 nm, 12 mW, 5 kHz) to deliver LLLT to the haemopoietic cell line U937. Fluences of 2.9 and 8.6 J/cm² increased superoxide and hydrogen peroxide production in the differentiated form of the cells but decreased DNA synthesis measured by the incorporation of tritiated thymidine. Addition of extracellular catalase abrogated the reduction in DNA synthesis caused by LLLT.

A group at MOE laser laboratory in Guangzhou, China has studied LLLT effects on HeLa cells illuminated with HeNe laser. They reported [19] that the fluorescent probe dichlorodihydrofluorescein was oxidized to its fluorescent product in a light fluence dependent manner, and at the same time Src tyrosine kinase was activated by ROS. Using a Src reporter based on fluorescence resonance energy transfer (FRET) and confocal laser scanning microscope, they visualized the dynamic Src activation in HeLa cells immediately after LLLT. The increase of Src phosphorylation at Tyr416 was detected by Western blotting. In the presence of vitamin C, catalase alone, or the combination of catalase and superoxide dismutase (SOD), the activation of Src by LLLT was significantly abolished. Cellular viability assay (formazan conversion) revealed that low doses (25 J/cm^2) promoted HeLa cell viability while high doses impaired viability [20].

Pal et al [21] used a HeNe laser to carry out LLLT of normal human skin fibroblasts. They administered either single cell illumination via a fiber optic nanoprobe to test for ROS production using DCDHF or bulk illumination to test for changes in cell proliferation as determined by cell counting. They found a dose dependent increase in cell proliferation with a maximum at 16 J/cm^2 . The dose-dependence of ROS generation was more complicated and depended on total fluence, irradiance and exposure time.

Alexandratou et al [22] used a 647-nm laser to illuminate single human fetal foreskin fibroblast cells on a confocal microscope delivering the small dose of 0.0015 J/cm^2 and found DCDHF oxidation reaching a maximum at 8 min post illumination. They also measured an increase in cellular pH, a periodic oscillation in cytosolic calcium and increase in mitochondrial membrane potential.

In our laboratory we have used DCDHF and dihydro-rhodamine 123 fluorescent probes to demonstrate production of ROS in mouse embryonic fibroblasts after LLLT [23]. We have so far used 810-nm and 980-nm lasers and have found a very broad dose response with fluences of 0.03 J/cm^2 starting to have a positive effect, 0.3 and 3 J/cm^2 having a maximum effect and 30 J/cm^2 having a reduced effect. The increase in ROS correlated with activation of NF- κ B transcription factor that could be prevented by addition of anti-oxidants, N-acetylcysteine and vitamin C.

4 CELLULAR RESPONSE TO OXIDATIVE STRESS

4.1 Oxidative stress

In respiring cells, a small amount of the consumed oxygen is reduced to alternative chemical species to the normal reduction product that is water. These highly reactive molecular entities are collectively called reactive oxygen species (ROS), and the main examples include superoxide anion ($\text{O}_2^{\bullet -}$), hydroxy radical (HO^{\bullet}), and hydrogen peroxide (H_2O_2). ROS are capable of causing oxidative damage to macromolecules leading to lipid peroxidation, oxidation of amino acid side chains (especially cysteine), formation of protein-protein cross-links, oxidation of polypeptide backbones resulting in protein fragmentation, DNA damage, and DNA strand breaks. High doses of ROS, which may be generated during chronic and acute inflammatory diseases or associated with toxic chemicals, radiation or other environmental stresses, are cytotoxic. Small amounts of ROS, produced as a consequence of electron transfer reactions in mitochondria, peroxisomes, and the cytosol, are scavenged by cellular defending systems including non-enzymatic and enzymatic antioxidants. A state of moderately increased levels of intracellular ROS is referred to as oxidative stress.

4.2 Cellular sensors for oxidative stress

Because of the ubiquitous nature of oxidative stress and the damaging effects of ROS, cells respond to these adverse conditions by modulation of their antioxidant levels, induction of new gene expression, and protein modification [24]. The homeostatic modulation of oxidant levels is a highly efficient mechanism that appeared early in evolution, allowing all cells to tightly control their redox status within a very narrow range. There are numerous molecular sensors within cells that can detect ROS by becoming oxidized and the new chemical structures formed initiate signal transduction pathways that can lead to a cascade of reactions [25]. Figure 2

graphically illustrates the range of ROS sensors, signal transduction intermediates and transcription factors that have been reported to govern cellular response to oxidative stress. Transcription factors are proteins that can translocate from the cytosol to the nucleus where they can bind to specific sequences of DNA (consensus binding site or response element) and thereby controls the transfer (or transcription) of genetic information from DNA to RNA. Transcription factors perform this function alone, or with other proteins in a complex, by promoting (as an activator), or blocking (as a repressor) the recruitment of RNA polymerase (the enzyme which activates the transcription of genetic information from DNA to RNA) to specific genes. There are approximately 2600 proteins in the human genome that contain DNA-binding domains and most of these are presumed to function as transcription factors.

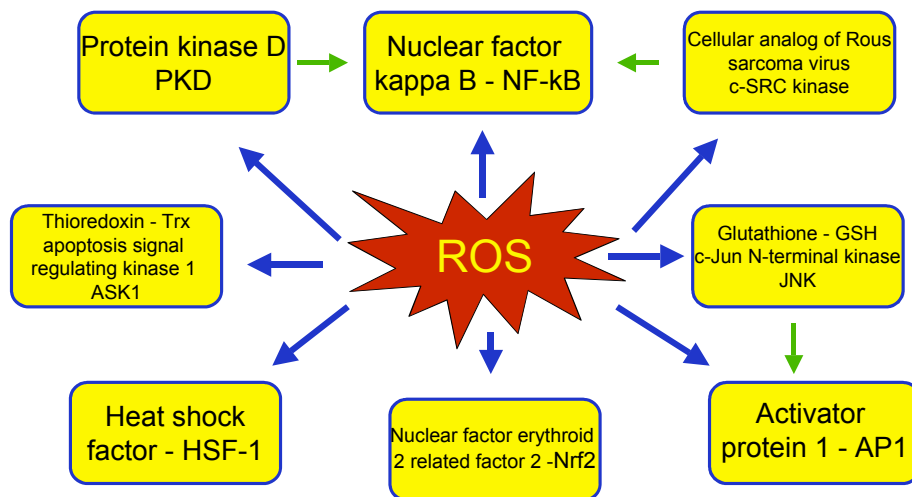


Figure 2. Schematic diagram of cellular sensors, signal transduction pathways and transcription factors that govern the response of cells to ROS

While the entire signal transduction cascades and transcription factors involved in the cell response to ROS and oxidative stress remains to be completely worked out, some facts are known. Cysteine residues do not form disulfide bonds, unless intracellular redox balance is tilted toward oxidant stress. The formation of disulfide bonds is capable of altering both conformation and activity of a number of enzymes, most notably of phosphatases. These enzymes usually restrict the activity of protein kinases. Inactivation of a specific phosphatase by oxidant stress results in prolonged activity for the kinases that it controls in a specific cell type. Prolonged activity of specific kinases, in a cell, means that particular intracellular signal cascades are increasingly activated. Such alterations in the intracellular signal cascades, which proceed through successive phosphorylations of particular kinases that operate on a pathway, culminate in phosphorylation of proteins in many cell compartments, such as mitochondria or nucleus. This modification of specific regulatory proteins can result in a number of changes, ranging from ionic signals to wide alterations in patterns of gene expression. As a consequence, a cell may change its rate of proliferation, or die, depending on the signal networks that it operates. An intracellular oscillation of oxidant levels has been previously experimentally linked to maintenance of the rate of cell proliferation [26].

Activator protein 1 (AP-1) is a transcription factor which is a heterodimeric protein composed of proteins belonging to the c-Fos, c-Jun, ATF and JDP families. It regulates gene expression in response to a variety of stimuli, including cytokines, growth factors, oxidative stress, and bacterial and viral infections. AP-1 in turn controls a number of cellular processes including differentiation, proliferation, and apoptosis. AP-1 upregulates

transcription of genes containing the 12-O-tetradecanoylphorbol 13-acetate (TPA) DNA response element (TRE; 5'-TGAG/CTCA-3) [27]. AP-1 binds to this DNA sequence via a leucine zipper [28].

4.3. NF- κ B activation

The Rel/NF- κ B family of transcriptional factors regulate expression of numerous cellular and viral genes and play important roles in immune and stress responses, inflammation, and apoptosis [29, 30]. It is thought that NF- κ B activity is regulated by the intracellular ROS levels, but the molecular mechanism involved in this regulation remains to be elucidated [31, 32]. The NF- κ B transcriptional factors are composed of homodimers or heterodimers of Rel proteins, which are characterized by the presence of a Rel homology domain (RHD) [33] that interacts with inhibitory proteins, members of the I κ B family. As a consequence of binding to cytoplasmic I κ Bs, the nuclear localization signal of the NF- κ B dimer is masked and NF- κ B is sequestered in the cytoplasm.

In response to proinflammatory cytokines such as tumor necrosis factor (TNF) and interleukin-1 (IL-1), bacterial lipopolysaccharide (LPS) or viral double-strand RNA (dsRNA), the I κ Bs are rapidly phosphorylated at two specific serine residues located at their N2-terminal region (Ser-32 and Ser-36 for I κ B α , and then undergo ubiquitination and proteolysis by the 26S proteasome, resulting in release and translocation of NF- κ B to the nucleus, where it activates transcription of specific target genes. The cytokine-induced phosphorylation of I κ B is a prerequisite for its degradation and subsequent NF- κ B activation because substitutions of those two serines with alanine residues render I κ B resistant to degradation, and the expression of such mutant forms of I κ B result in suppression of NF- κ B activation.

A large TNF-inducible cytoplasmic protein kinase complex that is able to phosphorylate I κ B α and I κ B β on the appropriate Ser residues was purified and two subunits were identified as two related protein kinases of molecular mass 85,000 and 87,000, called I κ B kinase (IKK) α (IKK1) and IKK β (IKK2), respectively [34, 35]. Both IKK α and IKK β are rapidly activated by cytokines, with kinetics matching those of I κ B phosphorylation and degradation. Expression of a catalytically inactive IKK α or IKK β mutants blocks cytokine-induced I κ B α degradation and NF- κ B activation, suggesting that IKK α and IKK β are important for induction of NF- κ B activity by cytokines [36].

In addition to IKK α or IKK β , another *bona fide* subunit of the large IKK complex was found to consist of two additional polypeptides (IKKg1 and IKKg2), which represent differently modified forms of the same protein IKKg [37]. The importance of IKKg was revealed by the reduction in cytokine-induced activity of IKK and degradation of I κ B α caused by expression of an antisense IKKg construct, which reduced IKKg expression by ~50%. A mouse homologue of IKKg was named NEMO (NF- κ B essential modulator) [38]. As described above, NF- κ B-activating stimuli that are also able to induce IKK activity include cytokines (TNF and IL-1), PMA, LPS, dsRNA, the HTLV transactivator protein Tax [39], and ionizing radiation [40]. In certain cases, however, NF- κ B activation does not seem to involve I κ B phosphorylation by IKK or even I κ B degradation. Short-wavelength UV (UV-C) light activates NF- κ B in certain cell types concomitantly with I κ B degradation. Pretreatment of cells with proteasome inhibitors blocked I κ B α degradation and NF- κ B activation induced by UV radiation, indicating that I κ B α degradation is required [40]. However, neither IKK activation nor the phosphorylation of I κ B α on Ser-32 and Ser-36 was observed to occur after UV-C irradiation [41].

4.4 ROS-induced activation of NF- κ B

The direct evidence that ROS level may be able to regulate NF- κ B was provided by frank exposure of cells to H₂O₂. In certain cell types, such as Wurzburger subclone of T cells, L6 skeletal muscle myotubes, human breast MCF-7, and 70Z/3 pre-B cells, H₂O₂ was shown to be an effective inducer of NF- κ B activation [42, 43]. Several groups have reported that H₂O₂ can also induce NF- κ B activation in HeLa cells, albeit to quite a different extent [44]. H₂O₂-induced NF- κ B activation is highly cell type dependent and therefore H₂O₂ is unlikely to be a general mediator of NF- κ B activation [45]. Intracellular level of reduced glutathione (GSH), which may differ from one cell type to another, may be crucial for H₂O₂-induced NF- κ B response. GSH is the

major intracellular thiol and ROI scavenger [46]. N-acetyl-L-cysteine (NAC) is a nontoxic compound that protects cells from oxidative damage [47]. It provides a precursor for GSH synthesis and can also react directly with ROI.

4.5 Activation of NF- κ B by LLLT

We have shown that NF- κ B can be activated in mouse embryonic fibroblasts by modest exposure of the cells to 810-nm or 980-nm laser light [23]. This was accomplished by isolating the MEF cells from transgenic mice (HLL mice) expressing *photinus* luciferase controlled by an NF- κ B-dependent promoter (5' human immunodeficiency virus-1 [HIV-1] long terminal repeat) [48]. The extent of NF- κ B activation could therefore be easily measured by luminescence assay after various laser exposure regimens [49]. We found that the degree of NF- κ B activation correlated well with the amount of ROS produced as described in section 3. We therefore propose that an important mechanistic pathway operating in LLLT is the activation of NF- κ B via low level laser induced mitochondrial ROS. It should be noted that as yet it is uncertain whether laser illumination is required for ROS generation and NF- κ B activation, or whether non-coherent LED light would work equally well.

5 RESULTS OF LLLT-MEDIATED ACTIVATION OF NF- κ B

In order to test whether LLLT-mediated activation of NF- κ B is a reasonable hypothesis that could explain the observed effects of LLLT on cells and tissues, we should examine the gene products that are expressed when NF- κ B binds to its various promoter elements. NF- κ B is one of the most prolific transcription factors operating in mammalian cells and more than one hundred and twenty genes are known to have NF- κ B responsive elements. Figure 3 shows many of the NF- κ B responsive genes divided into groups. Groups of genes are classified under headings for (a) anti-apoptosis proteins including inhibitors of apoptosis and Bcl-2 family members; (b) pro-proliferation proteins including growth factors, proto-oncogenes and cyclins; (c) adhesion molecules; (d) genes involved in adaptive immunity; (e) proteins involved in the acute phase response; (f) pro-inflammatory genes; (g) many cytokines and chemokines; (h) anti-oxidant genes such as superoxide dismutase and ferritin heavy chain. Activation of anti-apoptotic genes explains the tendency of LLLT to prevent cell death from noxious stimuli such as cyanide, tetrodotoxin, methanol [50, 51]. Activation of pro-proliferation genes explains many reports of increased cell numbers and increased viability assays in vitro after LLLT [17, 52, 53]. Activated fibroblasts can also synthesize more collagen in response to LLLT [54]. Activation of genes for adhesion molecules explains Karu's results on increased adhesion to glass of HeLa cells after LLLT [55] and also studies that show increased migration of fibroblasts to "heal" in vitro wounds [56]. The activation of antioxidant genes by NF- κ B explains the apparent paradox of LLLT being pro-oxidant in the short term but antioxidant in the long term [57]. The main apparent paradox that remains to be explained is the clear pro-inflammatory nature of many NF- κ B target genes, and the broadly accepted anti-inflammatory of LLLT especially in clinical studies but also in animal models.

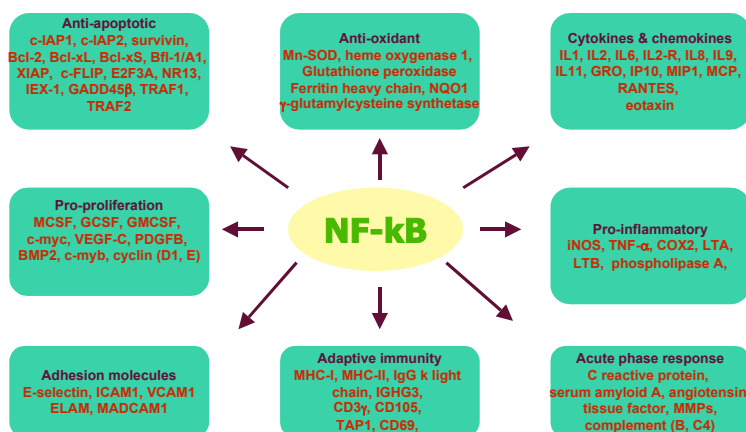


Figure 3. Schematic diagram of NF- κ B target genes divided into groups

In conclusion we believe that activation of NF- κ B and possibly activation of other redox-sensitive transcription factors explains many (if not most) of the observed responses of cells to LLLT in vitro, and is likely to play a role in the response of animals and patients to LLLT for both experimental and clinical indications and diseases.

6 ACKNOWLEDGEMENTS

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