Review

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Targeting the pain, inflammation and immune (PII) axis: plausible rationale for LLLT

Schmerz, Entzündung und Immunantwort: Eine plausible Begründung für die LLLT

Abstract: Low-level laser therapy (LLLT) has been used in many clinical contexts. Although its precise mechanism is unclear, its current use is based predominantly on its non-invasive nature and popular patient acceptance. This review attempts to provide a framework for the clinical disease-disorder states focusing on the etiopathology namely; pain, inflammation and immune response termed the PII axis. Following a brief introduction, the literature on the ability of LLLT to modulate the PII axis in specific disease states is reviewed. The triad of critical parameters for LLLT namely, the dose, the biological context and the mechanism are highlighted. This work suggests that LLLT could be a potent primary interventional modality when used to specifically target the PII axis in clinical disease management.

Keywords: low-level laser therapy; pain; inflammation; immune response.

Zusammenfassung: Die Low-Level-Laser-Therapie (LLLT) ist in vielen klinischen Zusammenhängen verwendet worden. Obwohl die genauen Mechanismen der LLLT noch unklar sind, beruht ihr aktueller Einsatz vor allem auf dem minimal-invasiven Charakter der Methode und der hohen Patientenakzeptanz. Der vorliegende Review-Artikel nähert sich dem Thema ausgehend vom Krankheitsverlauf verschiedener klinischer Erkrankungen und Störungen und fokussiert hierbei auf die Schmerz-Entzündungs-Immunantwort-Achse (pain, inflammation, immune response – PPI – axis). Nach einer kurzen Einführung wird basierend auf der aktuellen Literatur diskutiert, inwieweit es möglich ist, mittels LLLT die PII-Achse in bestimmten Krankheitsstadien zu modulieren. Die Triade kritischer Parameter für die LLLT, nämlich die Dosis, der biologische Kontext und der Mechanismus werden besonders herausgestellt. Im Ergebnis liegt die Vermutung nahe, dass die LLLT eine potente Interventionsmethode im Disease-Management darstellt, wenn sie zielgerichtet auf die PII-Achse ausgerichtet ist.

Schlüsselwörter: Low-Level-Laser-Therapie; Schmerz; Entzündung; Immunantwort.

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1 Introduction

The field of photomedicine encompasses a wide range of uses including photodynamic therapy (usually dye-assisted laser destruction), phototherapy (using UV and visible light sources), surgical lasers (high energy lasers as surgical cutting-coagulation tools) and low-level laser therapy (LLLT). This review will focus on the latter LLLT that is also referred to in the literature as “low intensity laser” or “cold laser” or “soft laser”. While LLLT is a medical subject heading (MeSH) term, it is still not precisely clear what the terms “low” and “level” refers to [1]. Given the recent popularity of light sources, especially LEDs, the term “light” has often been appropriately substituted for “laser”. In our experience, the critical parameter is the dose (energy, power and time) and this varies with the clinical-biological context [2]. The biological effects of the LLLT encompass both stimulatory and inhibitory biological effects collectively termed “photobiomodulation” [3]. The clinical effectiveness of LLLT applications and its mechanism of action are still controversial and extensive cellular, animal and human studies need to be done to well-establish the safety of use of LLLT. This review addresses some of the most popular uses of LLLT in current clinical management. The review

is outlined in three sections: first an introduction to the connections between pain, inflammation and immune response, termed the PII axis, is presented. The second section overviews the evidence for LLLT specifically targeting the PII axis in mediating its therapeutic effects. In the final section, the literature is reviewed in each condition supporting the use of LLLT for therapy. It should be pointed out that LLLT is currently used as an adjuvant in combination with physical or pharmacological therapy. The primary goal of this review is to provide an integrated view for the use of key parameters of LLLT namely; dose, contexts and mechanisms based on its ability to primary modulate the PII axis for therapy.

2 The nexus of pain, inflammation and immune response – PII axis

The defense mechanism evolved by the body to protect against damage by deleterious agents including physical injury and microbial infection involves a complex set of biochemical and cellular phases constituting the immune system. Broadly, the immune system is divided into innate immune response that is generic and non-specific to specific damage agents; while a more tailored, agent-specific response is part of the adaptive immune response. In order to provide a broad conceptual framework to assess the efficacy of LLLT in clinical disease management, we propose an integrated overview of the disease etiopathology termed the pain, inflammation and immune or the PII axis (Figure 1). The major purpose of proposing this conceptual outline is to present the combinatorial "causal" agents and "effector" mediators that have been demonstrated in individual diseases disorders. Further, this provides the rationale to highlight the literature showcasing the ability of LLLT to specifically modulate these pathways. It is hoped that this review will also highlight specific biomarkers or disease indicators that could be assessed in future LLLT studies to establish their efficacy with regards to various clinical disease-disorder states.

Figure 1 Depiction of the two effector processes of the immune response, pain and inflammation that form the etiopathology of many common clinical disease-disorder states termed the PII axis.

2.1 The effector process: inflammation

Inflammation is a key component of the innate response and is the protective response involving a complex reaction in vascularized connective tissue to rid the host of both the causes of cell injury and its associated consequences [4]. It is divided into two patterns – acute and chronic inflammation. Acute inflammation is the immediate response to an injurious agent. It involves vascular and cellular events. Vascular changes begin shortly after injury and occur in the following order: transient vasoconstriction followed by vasodilation that increases blood flow resulting mainly from arteriolar dilation and opening of capillary beds, slowing of circulation or stasis due to increased permeability of microvasculature and finally, leukocytic margination. The hallmark of acute inflammation is increased vascular permeability leading to the escape of protein rich fluid into interstitial tissues termed edema. Cellular events involve adhesion of leukocytes (initially predominantly neutrophils) to the endothelium and then transmigration or diapedesis across the endothelium to the interstitial tissues and migration towards the site of injury by a process called chemotaxis, followed by phagocytosis of the injurious agent. Chemotactic agents can be both endogenous, e.g., complement component 5a, leukotriene B4 (LTB4), interleukin 8 (IL-8), and exogenous (most common are bacterial products). During chemotaxis and phagocytosis, activated leukocytes may release products into the extracellular matrix that can lead to significant tissue damage. Some of the important products released by neutrophils are a) lysosomal enzymes, b) oxygen derived active metabolite, and c) products of arachidonic acid metabolism, including prostaglandin and leukotrienes. Acute inflammation can have any one of the four outcomes: a) complete resolution or healing, b) abscess formation, c) healing by connective tissue replacement or scarring, and d) progression to chronic inflammation.

Chronic inflammation, on the other hand, is of longer duration and is characterized by the presence of mononuclear cells (macrophages, lymphocytes, and plasma cells), tissue destruction and attempts at healing by connective tissue replacement by angiogenesis (proliferation of blood vessels) and fibrosis. The functional transformation of the monocytes to macrophage at the sites of tissue damage is a main feature of chronic inflammation. Macrophages are activated by signals such as the cytokine interferon-gamma

(IFN-γ) (from activated T-cell), endotoxin, fibronectin and chemical mediators release products which can cause tissue injury and fibrosis. Other cell types involved in chronic inflammation are lymphocytes, plasma cells, mast cells and eosinophils. Tissue destruction is one of the hallmark features of chronic inflammation, which itself can perpetuate the inflammatory cascades, both acute and chronic, through multiple effector pathways.

2.2 The alarm system: pain

The body has developed a damage sensing alarm signal – pain – to indicate the presence and often persistence of a damage inciting agent. There are many well characterized pain inciting agents with well-elucidated physicochemical mediators as well as psycho-social perception pathways that have been well elucidated. This review focuses on the ability of LLLT to modulate the peripheral – local and regional – mediators of pain inciting agents. Pain is broadly classified based on its type and character as acute or chronic, peripheral or central, nociceptive or neuropathic pain. One school of thought believes that the origin of all pain is related to inflammation [5]. The biochemical mediators of inflammation can stimulate local pain receptors and nerve terminals leading to hypersensitivity in the area of injury. They can also lead to pain hypersensitivity in neighboring uninjured areas (secondary hyperalgesia) due to diffusion of inflammatory mediators and increased nerve excitability of the spinal cord. Some mediators can act directly on membrane ion channel proteins and increase permeability and cell excitability.

Nerve impulses reaching the spinal cord stimulate the release of inflammatory protein substance P. Substance P along with other inflammatory proteins like calcitonin gene-related peptide (CGRP), neurokinin A and vasoactive intestinal peptide (VIP) removes magnesium-induced inhibition enabling excitatory proteins, such as glutamate and asparate, to stimulate specialized spinal cord N-methyl-D-aspartic acid (NMDA) receptors. This leads to magnification of nerve impulses and pain stimuli that arrive in the spinal cord from the periphery. The activation of motor nerves leads to increased muscle tension; this further leads to release of inflammatory mediators and subsequent excitation of pain receptors in muscles, tendons and joints and hence more nerve traffic and increased muscle spasm. Thus, continuous abnormal spinal reflex transmission due to local injury or abnormal postural habits leads to a vicious circle of muscle spasm and pain. The C-fiber stimulation of transmission pathways in spinal cord also leads to increased release of inflammatory mediators in the spinal cord. Therefore, activation of pain receptors, transmission and modulation of pain signals, neuroplasticity, and central sensitization appear to be one single continuum of inflammation and inflammatory-immune response [5]. Every pain syndrome has its unique profile with respect to the particular biochemical mediator of inflammation present and the amount; but can vary in the same patient and from one patient to the other. Many of the classical biochemical pain mediators are well characterized, such as prostanoids, kinins, serotonin, histamine, cytokines and neuropeptides, among others. Roles for reactive oxygen species (ROS), altered pH and adenosine triphosphate (ATP) in mediating pain have also been described. The latter have been shown to be directly modulated by LLLT.

- *Prostanoids* (prostaglandins, leukotrienes, eicosanoids) – key mediators of inflammatory hyperalgesia. They sensitize peripheral nerve terminals reducing their activation threshold causing localized secondary hyperalgesia. They act via a number of receptors coupled with second messengers, but the EP receptor for prostaglandin E (PGE-2) and IP receptor for prostaglandin I (PGI-2) are the most important receptors for their effect on sensory neurons [6]. Recently, receptor subtype EP3 has been identified in the majority of small sensory neurons. Studies have shown that PGE-1 and PGI-2 have increased the activity of nociceptors directly, whereas PGE-2 stimulated the release of substance P from sensory neurons in culture. These effects may have been due to increase in sodium conductance. Intradermal injection of LTB4 or (8R,15S)-Dihydroxyicosa-(5E-9,11,13Z)-tetraenoic acid also leads to the decrease in nociceptive threshold [7, 8].
- *Kinins* – directly stimulate pain receptors in skin, joint and muscle and can sensitize them to heat and mechanical stimuli. There is strong synergism between actions of bradykinin and other pain generating mediators like prostaglandin and serotonin. Bradykinin through protein kinase C (PKC) leads to excitation of afferent fibers due to an increase in membrane ion permeability, mainly to sodium ions. This depolarization leads to calcium influx causing the release of substance P and activation of phospholipase C. Also, prostaglandins and bradykinin, inhibit the slow fibers after hyperpolarization by stimulating adenosine 3′,5′-cyclic monophosphate (cAMP) formation, allowing neurons to fire repetitively.
- *Serotonin* – monoamine neurotransmitter that is abundant in the gastrointestinal tract, platelets and central nervous system. It can directly excite sensory

neurons by increasing sodium permeability via 5-HT3 receptor activation.
- *Histamine* – histamine H1 receptor activation on sensory neurons leads to an increase in membrane calcium permeability, which leads to the release of sensory neuropeptides, prostaglandins and 5-hydroxyeicosatetraenoic acid from endothelial cells leading to hyperalgesia and other pro-inflammatory affects.
- *Cytokines* (IL-1β, IL-6, IL-8, IL-10, TNF-α) – can cause hyperalgesia via various indirect mechanisms like increased production of prostaglandin or increasing the expression of bradykinin or nerve growth factor (NGF) receptors. Tumor necrosis factor-alpha (TNF-α) release leads to increased production of prostaglandin which further leads to increased production of glutamate and thus increase in nerve cell communication. It also leads to excitation of pain receptors and stimulation of specialized nerves such as the C-fibers and Aδ-fibers.
- *Neuropeptides* (neurokinins and neurotropins) – during inflammation neurokinins substance P and neurokinin A (NKA) contribute directly and indirectly to neurogenic inflammation and hyperalgesia in periphery and excitability in dorsal horn cells of spinal cord associated with transmission of pain signals. Neurokinins can also reduce potassium permeability and can directly depolarize sensory neurons. During inflammation, neurotrophins like NGF increase the synthesis of neurokinis and CGRP. Substance P also leads to an increase in TNF-α production.
- *Free radicals and reactive oxygen species* – Hydrogen peroxide has been shown to enhance the effects of bradykinin and PGE-2. The intra-dermal injection of nitric oxide (NO) induces a delayed burning pain [9]. During inflammation or nerve injury, an inducible and calcium dependant form of nitric oxide synthase (NOS) leads to an increase in NO synthesis. Inducible NOS (iNOS) has a role in upregulation of cyclooxygenase (COX) activity and hence the production of pro-inflammatory prostanoids [10]. NO may also alter the response of sensory neurons to bradykinin and contributes to hyperalgesia by increasing sensitization to central and peripheral stimuli.
- *Altered pH (protons)* – Change in tissue pH due to inflammation generates positively charged subatomic particles called protons. They are associated with inflammatory hyperalgesia and pain-discomfort due to hypoxia observed during muscle exercise. Intradermal injection of acidic solution leads to sharp stinging pain due to direct activation of nociceptors and enhancing the effects of other inflammatory mediators.
- *Adenosine triphosphate* – can activate sensory neurons and increase their permeability to cations. Adenosine formed on breakdown of ATP, also provokes pain and hyperalgesia due to stimulation of adenosine A2 receptors which are coupled with cAMP. The production of cAMP and decrease in potassium ion permeability accounts for hyperexcitability of sensory neurons.

2.3 Damage-associated molecular patterns

Damaged-associated molecular patterns (DAMPs) play an important role in signaling to the immune system [11, 12]. Inflammatory response occurs when pattern recognition receptors (PRRs) on the surface of innate immune cells detect the release of DAMPs from injured tissue in the absence of microbial invasion [13]. For example, ROS in high concentrations can cause tissue damage that can induce DAMPs which are recognized by PRRs [14]. Toll-like receptors are activated by DAMPs, which induces inflammatory gene expression in an effort to mediate the repair of damaged tissue [15]. As a result, DAMPs have been thought to play a key role in inflammatory diseases, such as rheumatoid arthritis and oral mucositis, as they promote nuclear factor kappa B (NF-κB) signaling and upregulate the expression of pro-inflammatory cytokines [16]. Thus, DAMPs play important roles in both induction and perpetuation of a pro-inflammatory responses and have been linked to many autoimmune diseases.

3 Can the PII axis be modulated by LLLT?

Conventional approaches to managing pain and inflammation include the use of pharmacologic drugs, most commonly non-steroidal anti-inflammatory drugs (NSAIDs) [17]. However, NSAIDs generally only treat the symptoms of inflammation; they do not directly target the cause of disease and many of the negative side effects related to the long-term use of these drugs have been documented [18]. For example in neck pain, the standard treatment includes simple analgesics, NSAIDs, and physical therapy [19]. For disease where definitive treatment strategies are

still not well established, conventional treatments often depend on the severity of the disease and the patient's preference. In carpel tunnel syndrome for instance, options include splinting, local injection or oral intake of corticosteroids, vitamin B6 or B12, or NSAIDs [20]. When conservative treatments based on clinical symptom fail, another approach is to target the PII axis by either removing the putative sources via surgery or by inhibiting key inflammatory and immune pathways [21]. Some clinical examples are oral root planing and scaling in periodontitis or antimicrobials and steroids in acute pulmonary inflammation. Some newer therapeutic approaches attempt to inhibit the effectors of the PII axis focusing on cytokines, such as IL-1β, that amplify immune reactions and play an important role in mediating the interaction between immune cells and disease progression such as in type 2 diabetes [22, 23].

There are many reports on the clinical efficacy of LLLT modulating the PII axis. A number of *in vitro* and *in vivo* studies have shown that LLLT reduces inflammation and pain and promotes wound healing. Lavi et al. [24] observed an increase in hydrogen peroxide following laser irradiation and postulated that these ROS may be responsible for induction of cell processes. Karu and Kolyakov [3] have shown that cytochrome C oxidase, a key enzyme in mitochondrial function, absorbs at specific wavelengths and can mediate the ROS generation. Further, Eells et al. [25] have demonstrated that the competitive inhibition of cytochrome C oxidase in methanol intoxication can be reversed by LLLT establishing its key functional role. Bertolini et al. [26] found that laser irradiation at 830 nm with a dose of 4 and 8 J/cm^2, reduced pain in rats with sciatica. Immune cells appear to be strongly affected by LLLT. Dendritic cells are antigen-presenting cells that initiate and modulate inflammatory and immune response. Isolated bone-marrow dendritic cells were stimulated with infection mimicking agents, such as lipopolysaccharide (LPS) or cytosine-phosphorothioate-guanine (CpG) oligodeoxynucleotide, and then treated with laser irradiation (810 nm at doses of 0.3, 3, and 30 J/cm^2). It was demonstrated that LLLT down-regulated IL-12 secretion from both LPS and CpG-stimulated conditions and reduced NF-κB activation in reporter cells stimulated with CpG, suggesting that LLLT has an anti-inflammatory effect on activated dendritic cells [27]. In an *in vitro* experiment, human osteosarcoma cells (MG63) were exposed to LPS to induce an inflammatory response and then LLLT treatment with a 920 nm diode laser, energy density of 5 or 10 J/cm^2 for 2.5 or 5 s demonstrated a significantly lower expression of iNOS, TNF-α, and IL-1 after 12 h compared to the non-laser irradiated LPS-induced controls [28]. Mast cells, which play an important role in the movement of leukocytes, are also important in inflammatory response and wound healing. It was found that LLLT increases mast cell degranulation and contributes to the inflammatory phase of the wound healing process [29, 30]. The broad range of LLLT parameters in these treatment protocols and their results reflects our limited understanding of LLLT-mediated alleviation of pain and inflammation in these diverse clinical scenarios [31].

In a previous study, we observed the ability of LLLT (904 nm, 3 J/cm^2) to activate latent TGF-β1 in a human oral wound healing model [32]. In this study, a same patient control and experimental sites were assessed by histology and demonstrated better organization and rate of healing in the LLLT wounds. The LLLT groups were noted to have increased TGF-β1 expression compared to the same patient controls. Further, serum latent TGF-βs were noted to be activated following LLLT irradiation. TGF-β is not only a central wound cytokine but also a potent immune-modulator as well as distinct roles in various pathophysiological contexts based on the cellular context, dose and timing [33]. The ability of LLLT to activate TGF-β has significant clinical implications in the disease context, especially in its ability to modulate the PII axis. In another recent study, we have observed the ability of LLLT (810 nm, 0.3–30 J/cm^2) to modulate ATP synthesis and ROS generation via distinct mitochondrial pathways [34]. NF-κB plays a critical role in mediating the PII axis and is specifically a key player in integrating and determining the final biological outcomes following tissue damage. We used primary mouse embryonic fibroblasts derived from NF-κB reporter mice to specifically outline the ability of LLLT to modulate ROS generation and increase ATP synthesis leading up to the activation of the NF-κB response.

These studies represent distinct effects on the PII axis via modulation of potent extra-cellular cues and intracellular molecular mediators suggesting a causal mechanistic basis for the use of LLLT in clinical therapy (Figure 2).

There are bound to be other factors induced following LLLT that may play critical roles in specific clinical-biological contexts. The ability of LLLT to modulate pain opens up various interesting possibilities on its effects on peripheral nociceptive mediators such as prostanoids, kinnins, serotonins, histamines, neuropeptides and other cytokines. Further, the role of local and regional molecular mediators potentially modulating the central nociceptive perception pathways may also be interesting to explore in conditions such as described for LLLT in phantom limb syndrome [35]. As we appreciate the role of systemic, central nociceptive aspects of chronic pain syndromes, the ability of LLLT to alleviate pain and provide patient

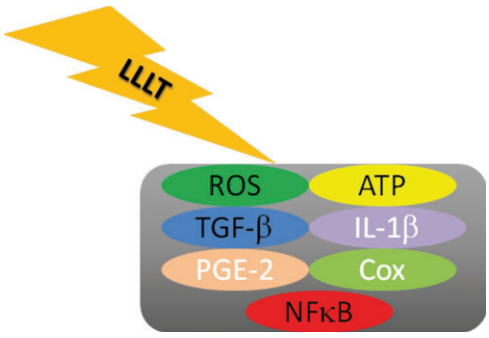

Figure 2 Some of the key molecular mediators of the PII axis shown to be modulated by LLLT indicating the rationale for its clinical use as well as their utility as disease biomarkers, black font are observations made in our studies.

relief should be documented by well-designed studies that evaluate and analyze these components. Further, while we appreciate there are specific DAMPs mediated induction and promotion of inflammatory states, it is as yet unclear if LLLT may modulate the same axis or mitigate these processes by acting on distinct components of the PII axis. These are all fascinating areas for future studies using objective clinical and laboratory endpoints such as objective disease biomarkers, functional imaging as well as patient responses and large population controlled clinical studies.

4 Clinical management with LLLT based on PII axis of diseases

Immune-mediated inflammatory diseases are thought to develop due to failures in the immune system that is either inappropriately directed (against self, autoimmune) or dysregulated. Inflammation is the protective pathophysiological response of the body to help prevent noxious damage and return to a homeostatic physiological state. But in scenarios of persistent stimuli or uncontrolled inflammatory reactions, this mechanism can turn pathological and will instead result in harm to the host. This final section of the review presents evidence for the use of LLLT in specific clinical contexts such as wound healing, acute and chronic inflammatory clinical disease states.

4.1 LLLT in wound healing

Wound healing is a dynamic process with an immediate goal to achieve tissue repair and homeostasis. It involves four overlapping phases – inflammation, vascularization, tissue formation and tissue remodeling. Tissue injury cause immediate formation of hemostatic plug; platelets and polymorphonuclear leukocytes entrapped in blood clot release a wide variety of inflammatory mediators which initiate coagulation cascade and attract the inflammatory cells enhancing the inflammatory response. Tissue formation involves closure of the wound area by reepithelization and formation of granulation tissue involving endothelial cells, macrophages and fibroblasts. Tissue remodeling involves synthesis, remodeling and deposition of structural extracellular matrix molecules completing the healing phases. The ideal outcome of wound healing would be complete reconstitution and restoration of function termed regeneration.

The role of LLLT in wound healing has been extensively reviewed [36, 37]. A meta-analysis study by Enwemeka et al. [38] showed the positive effects of LLLT on all three phases of tissue repair: a) inflammation such as mast cell proliferation and degranulation, b) cellular proliferation (fibroblasts, keratinocytes, osteoblasts, chondrocytes) and collagen synthesis, and c) tissue maturation through its positive effect on tensile strength of repaired tissue. A study by Medrado et al. [39] on rats treated cutaneous wounds with local application of 670 nm gallium-aluminum arsenide (GaAlAs) laser and observed the reduction in edema and inflammatory cells, increase in collagen and elastic fibers and increased proliferation of myofibroblasts as compared to untreated controls. The early effect of laser therapy is reduction in early edema. The study also showed early replacement of segmented leukocytes by mononuclear cells in the laser treated group as compared to controls. Another crucial difference noted between treated wounds and controls was the increase in fusiform cells expressing desmin and alpha smooth muscle actin in the treated group 72 h after surgery. This increased number of fusiform cells corresponds to the time the cutaneous wounds showed greatest reduction in diameter in treated group as compared to controls, indicating a direct effect of laser treatment. In the present study, though there were favorable changes for resolution of wound healing, but there was no reduction in cicatrization (scar formation) time. An *in vitro* study done by Haas et al. [40] on keratinocytes, found an increase in their motility following helium-neon (HeNe) irradiation, but observed no change in their proliferation and differentiation. Recently, Grossman et al. [41] demonstrated an increase in keratinocyte proliferation upon exposure with 780 nm continuous-wave diode laser. Bjordal et al. [42] summarized that local effects of LLLT occur in <24 h after first irradiation. They summarized

that the effects of LLLT on biochemistry of inflammatory process include reduction in levels of PGE-2, TNF, IL-1, COX-2 and plasminogen activator. Also, the effects of LLLT on cells and soft tissues include reduction in edema and hemorrhage formation, neutrophil influx, cell apoptosis and improved microcirculation.

Various studies have been done on the effects of LLLT on healing on the animal models. Stadler et al. [43] demonstrated an increase in cutaneous wound tensile strength on a diabetic mouse following irradiation with 830 nm diode laser. Contrary to these studies, a study done by Lowe et al. [44] observed no significant improvement on wound healing in mice expose to ionizing irradiation upon treatment with 890 nm laser. Similarly, Walker et al. [45] also found no changes in wound healing process in irradiation – impaired mice upon treatment with 660 nm GaAlAs laser. These negative results emphasize the need to pay close attention to the experimental models used. The specific use of ionizing radiation to produce the injury may have secondary deleterious cellular effects as has been well documented in the literature. Further, activation of specific growth factor and cytokine pathways, such as TGF-βs, by ionizing radiation may bias the healing milieu preventing the beneficial effects of non-ionizing LLLT. Similarly, a study done by Hunter et al. [46] on pigs demonstrated no hastening of wound healing on exposure with HeNe laser. Researchers argue that the healing mechanisms of animals like mice, rats and guinea pigs occur predominantly by contraction due to loose elastic skin and panniculus carnosis as compared to humans and pigs where healing is mainly by true reepithelization [36].

Studies on LLLT in humans were pioneered by Mester et al. [47] who found healing of chronic soft tissue ulcer upon treatment with ruby laser at 1–4 J/cm^2 energy density. They also found improvement in 70% of recalcitrant ulcers they examined, upon treatment with laser at approximately 4 J/cm^2. Schindl et al. [48] demonstrated improved healing in a persistent radiation ulcer upon exposure with HeNe laser at 31.5 J/cm^2. Another case report found an improvement in the healing of diabetic neuropathic foot ulcers following treatment with 670 nm diode laser along with oral antibiotics and dressing change [49]. A study done on 30 patients with diabetic microangiopathy, postulated that laser irradiation caused cytokine release which might be beneficial in the treatment of diabetic microangiopathy [50]. A study on humans by Pourreau-Schneider et al. [51] showed the early appearance of myofibroblasts in an intra oral area after the laser irradiation as compared to the control site. The myofibroblasts appeared within 48 h of laser application in treated sites, whereas the control site did not show these cells within at the same time. Contrary to the results in these studies above, there are reports of LLLT being inefficacious in certain clinical scenarios. Lundeberg and Malm [52] found no significant difference in the percentage of venous leg ulcer area healed upon treatment with an HeNe laser at 4 J/cm^2 as compared to the placebo. Similarly, Malm and Lundeberg [53] found no difference in the rate of healing of venous ulcers following exposure with 904 nm gallium arsenide (GaAs) laser. Lagan et al. [54] also observed no difference in wound healing rate or pain levels in patient with post-surgical wounds upon treatment with 830 nm GaAlAs laser.

4.2 LLLT in acute pulmonary inflammatory disease

In an acute pulmonary inflammatory model in rats, animals received saline (control), LLLT, LPS, LPS+LLLT or LPS + dexamethasone treatment. Rats exposed to LLLT (650 nm, 1.3 J/cm^2) after induction of inflammation by LPS after 1 h demonstrated significant down-regulation of pro-inflammatory cytokines TNF-α and IL-1β and inhibition of pulmonary edema and neutrophilic inflammation [55]. A similar study by Mafra de Lima et al. [56] found that LLLT (660 nm, 30 mW, 0.08 cm^2) can attenuate acute lung inflammation induced by intestinal ischemia and reperfusion in rats pre-treated with either anti-TNF-α or IL-10 antibodies by significantly down-regulating TNF-α and upregulating IL-10. TNF-α inhibitors are the standard treatment for rheumatoid arthritis (RA). Two controlled studies by Aimbire et al. [57] conducted on animals with induced lung injury, showed dose-dependent reduction in TNF-α expression following irradiation with a 650 nm GaAlAs laser.

4.3 LLLT in gingivitis and periodontitis

Gingivitis is the inflammation of the gingiva with redness, swelling and an increased tendency of the gingiva to bleed on gentle probing. Periodontitis is characterized by clinical attachment loss, deep pockets and crestal bone loss. The progression from health to gingivitis and periodontitis can be divided into four phases – initial, early, established and advanced. The initial lesion which occurs within 4 days of plaque accumulation involves an acute inflammatory response to plaque. The progression from gingivitis to periodontits is marked by change in T-cell to B-cell predominance [58]. The bacterial products (like LPS) and the inflammatory mediators from host derived immune response [like TNF-α, IL-1, PGE-2, IFN-γ, matrix

metalloproteinases (MMPs)] contribute to the pathogenesis of periodontal disease [59].

Since gingival fibroblasts are important in the late wound healing phase, their stimulation might help in the healing process. A study done by Kreisler et al. [60] used primary cells from gingival connective tissue explants that were irradiated with a 809 nm laser and noted an increase in the proliferation rate of these cells but noted a limited response leading them to conclude that multiple laser irradiation may be desirable for clinical benefits on healing. Tuby et al. [61] has shown an increase in expression of fibroblast growth factor by macrophages and fibroblasts upon irradiation with LLLT.

In an elegant study, Shimizu et al. [62] demonstrated inhibition of the production of PGE-2 and IL-1β in an *in vitro* study conducted on stretched human periodontal ligament cells derived from healthy premolars and irradiated with an 830 nm GaAlAs low power diode laser, concluding the role of low power laser in reducing pain accompanying tooth movement in orthodontic treatment. The study showed complete inhibition of production of PGE-2 in a dose-dependent manner, though the reduction of IL-1β was only partial. The study demonstrated down-regulation in the activity of IL-1β converting enzyme by laser irradiation; the enzyme cleaved IL-1β precursor to mature IL-1β. Since IL-1 is a powerful stimulator of PGE-2, and there is only partial inhibition in the production of IL-1, this study went on to show the down-regulation of COX activity that can inhibit PGE-2 production. Since PGE-2 and IL-1β play crucial roles in bone resorption, the LLLT may reduce bone resorption via inhibition of PGE-2 and IL-1β production. A controlled clinical pilot study done by Ozcelik et al. [63] on 20 patients with inflammatory gingival hyperplasia, demonstrated an improvement in epithelization and wound healing following gingivectomy and gingivoplasty procedures.

4.4 LLLT for carpal tunnel syndrome

Carpal tunnel syndrome (CTS) is an inflammatory disorder associated with compression of the median nerve at the wrist. Studies have shown increased expression of vascular endothelial growth factor (VEGF) and PGE-2 in the tenosynovium of CTS patients, which is thought to lead to thickening and play a role in the development of CTS [64]. Tucci et al. [65] found a significant increase in IL-6 and malionaldehyde bis-(diethyl acetal), and a five-fold elevation in PGE-2 in tissue samples from CTS patients compared to control tissues.

Clinical studies have investigated the effects of low power laser therapy for the treatment of CTS to harness the laser's anti-inflammatory effects and ability to improve microcirculation. A study by Shooshtari et al. [66] demonstrated significant improvement in clinical symptoms and hand grip in patients that were treated with laser irradiation at a dose of 9–11 J/cm^2 compared to those who received sham laser treatment. Chang et al. [67] also investigated the therapeutic benefits of laser irradiation at 9.7 J/cm^2 using an 830 nm diode laser on CTS patients for 2 weeks. The study demonstrated statistically significant improvements in grip strength and clinical symptoms after the 2-week follow-up; however there was no significant difference in nerve conduction studies between the treatment and control groups. In a more recent study, researchers demonstrated clinical improvement and pain reduction in CTS patients treated with LLLT using a 904 nm GaAs laser at 6 J/cm^2, with significant benefits persisting for up to 6 months [68]. As in other clinical scenarios, the clinical evidence for using LLLT to treat CTS is also inconsistent. Tascioglu et al. [69] concluded that there were no significant improvements in CTS symptoms or nerve conduction based on electroneuromyographic and ultrasonographic testing for patients treated with LLLT using an 830 nm GaAlAs diode laser at 6 J/cm^2 and 3 J/cm^2 compared to untreated controls.

4.5 LLLT in rheumatoid arthritis

RA is an autoimmune disease provoked by CD4+ T-cells, in particular IL-17 producing helper T (Th17) cells, that results in local joint inflammation and the development of arthritis [70, 71]. IL-17 plays a role in the migration of innate immune cells and the production of other pro-inflammatory cytokines, control of extracellular pathogens, and induction of matrix destruction. IL-17 targets osteoblasts and chondrocytes, releasing receptor activator for NF-κB ligand (RANKL), MMPs, and osteoclastogenesis, leading to bone erosion and cartilage damage and resulting in RA [72]. TGF-β1 is another important regulatory molecule in T-cells that has the ability to exacerbate inflammatory effects in collagen-induced arthritis RA models due to the increased production of IFN-γ and TNF-α. Due to the pleiotropic effects of TGF-β1, there is also evidence that this molecule can also play an anti-inflammatory role as well [73]. Pro-inflammatory cytokines, such as IL-1, IL-6, and TNF-α, contribute to the development and progression of RA in animal models [71].

Clinically, low power laser irradiation has been used for the relief of pain in RA. LLLT has also been tested for

anti-inflammatory effects in RA models. In a collagen-induced arthritis rat model, researchers subjected rats to LLLT (830 nm, GaAlAs diode, 7.64 J/cm^2, 20 min, 3 times a week for 2 weeks) and concluded that LLLT decreases the synthesis of chemokine (C-C motif) ligand 2 (CCL2) in RA synovial membrane tissues [74]. In a collagenase-induced tendinitis rat model, rats were subjected to LLLT (780 nm, 75 s, 7.7 J/cm^2) for 12 h and 7 days post-induction. The LLLT group had significantly less IL-6, COX-2, and TGF-β expression compared to the control group [75]. In a zymosan-induced inflammatory arthritis model, Castano et al. [76] suggest the importance of LLLT time over other irradiation parameters such as irradiance and fluence. Although time is a critical parameter due to physical attributes of the target tissue and cumulative rate constants of routine biological reactions, the significance of both irradiance and fluence must also be carefully appreciated [77].

4.6 LLLT in myofascial and musculoskeletal disorders

Chronic myofascial pain syndrome (MPS) is a condition characterized by regional pain and muscle tenderness and presence of hypersensitive nodules called myofascial trigger points. Local tenderness associated with acute muscle pain is caused by peripheral sensitization of local muscle nociceptors. Nociceptive terminals in muscles have great numbers of receptors in their membranes including receptors for bradykinin, serotonin, altered pH (protons) and prostaglandins. Also, continuous activation of muscle nociceptors by these inflammatory mediators or other endogenous substances can lead to central sensitization of dorsal horn cells. The continuous presence of these mediators released from damaged tissues and other biochemical mediators may be responsible for persistent pain conditions like MPS [78]. Chronic musculoskeletal pain, including back and neck pain, are a class of inflammatory pain syndromes that commonly result from injury to the muscle, disk, nerve, ligament or facet joint with a subsequent inflammatory reaction. Research suggests that back and neck pain is associated with the release of proinflammatory cytokines, in particular TNF-α, which upregulates prostaglandin, NO, and phospholipase A2 [79].

A growing amount of literature suggests that muscle regeneration requires cell proliferation, migration, and differentiation and is regulated by growth factors and cytokines. Increased local presence of proinflammatory cytokines, in particular TNF-α, IL-1β, and IL-6, and oxidative stress were associated with muscle wasting [80, 81]. In particular, studies suggest that enhanced levels of TNF-α lead to skeletal muscle atrophy in conditions such as chronic heart failure, cancer, AIDS, and cachexia induced by bacteria. Recent studies have also shown evidence that the activation of NF-κB leads to skeletal muscle wasting and the inhibition of signaling pathway prevents the loss of skeletal muscle mass [82].

In a study by Mesquita-Ferrari et al. [83] researchers investigated the effect of LLLT on the expression of TNF-α and TGF-β in the tibialis anterior of Wistar rats with a cryoinjury. The rats were divided into the following experimental groups: control, cryoinjury without LLLT group, and cryoinjury with LLLT [aluminium gallium indium phosphide (AlGaInP) laser, 660 nm, 5 J/cm^2, 10 s, 3 times per week]. Compared to the control, LLLT was able to down-regulate TNF-α and TGF-β, demonstrating the ability for LLLT to modulate cytokine expression and contribute to muscle repair. In a recent study, Luo et al. [84] studied the effects of LLLT (635 nm GaAlAs laser, 7.0 mW, 17.5 mW/cm^2) on skeletal muscle repair by measuring ROS generation and expression of insulin-like growth factor 1 (IGF-1) and TGF-β1 in the gastrocnemius muscles of adult male Sprague-Dawley rats following contusion. The results demonstrated that LLLT promoted the regeneration of muscle, reduced scar formation, enhanced muscle superoxide dismutase activity, and decreased muscle malondialdehyde levels. LLLT was found to modulate the expression of IGF-1 and TGF-β1, which play an important role in the repair process. LLLT upregulated IGF-1 on days 2, 3 and 7 following after injury while down-regulating expression on day 21 and 28. In contrast, LLLT down-regulated TGF-β1 levels on day 3 and 28 after injury, but upregulated it at day 7 and 14 [84].

To better understand the effects of LLLT on the collagen component of the extracellular matrix during skeletal muscle remodeling, a study by de Souza et al. [85] used LLLT (660 nm AlGaInP, 20 mW, 0.5 mW/cm^2) to treat rats following cryoinjury. This study revealed that at day 7, there was a significant reduction in myonecrosis associated with angiogenesis and significant upregulation of type I and III collagen in the laser-treated group compared to cryoinjured, non-laser-treated group, suggesting the ability for LLLT to stimulate the regenerative and fibrotic phases of skeletal muscle repair.

4.7 LLLT in chronic back pain

A study by Gur et al. [86] on 75 patients demonstrated the beneficial effects of low power laser therapy (GaAs laser)

in reducing pain and functional disability in chronic low back pain. In a separate study, they conducted a prospective, double-blind, randomized and controlled trial on patients with chronic MPS [87]. They concluded that there was improvement in functional ability, quality of life and pain relief in MPS patient who received short-period application of LLLT [87].

4.8 LLLT in neck pain

LLLT has also been used for the management of other types of musculoskeletal pain and MPS, including neck pain. In 2006, Chow et al. [88] conducted a double-blind, randomized, placebo-controlled study on 90 patients with chronic neck pain using a 300 mW, 830 nm laser consisting of 14 treatments over 7 weeks. Based on the visual analogue scale (VAS) for pain and outcome measures, the researchers concluded that LLLT significantly reduced pain in the active group compared to the placebo group over 3 months. More recently, the efficacy of LLLT (830 nm GaAsAl laser, 450 mW) in treating MPS in the neck was tested in a double-blind, randomized controlled trial with 64 patients. Using the VAS assessment as the primary pain outcome measure, after 4 weeks there was no statistically significant improvement in neck pain compared to the placebo group [89].

4.9 LLLT in tendinitis

Among the major effectors of inflammation are MMPs that mediate matrix turnover. The balance of MMPs and their inhibitors play an important role in tendon matrix morphology, and an imbalance can often lead to tendinitis. The expression of pro-inflammatory cytokines, such as IL-1β and TNF-α, can stimulate the synthesis of MMPs, which directly affects tendon growth, remodeling, and healing. Tendinitis and other tendinopathies have increased the expression of MMP-1, MMP-9, and MMP-13, along with decreased type II collagen expression due to degradation of collagen during inflammation. A recent study by Marcos et al. [18] treated collagenase-induced Achilles tendinitis rats with two doses of LLLT (810 nm, 35.71 J/cm^2, 10 s and 107.14 J/cm^2, 30 s). Following laser irradiation, it was found that LLLT significantly down-regulated COX-2, TNF-α, MMP-3, MMP-9, and MMP-13 gene expression, as well as PGE-2 compared to rats without LLLT. These results suggest that LLLT has the ability to reduce short-term tendon inflammation and the potential to effectively treat Achilles tendinitis.

4.10 LLLT in chronic vascular obstructive disease

Hsieh et al. [90] used a continuous 660 nm GaAlAs diode laser at a dose of 9 J/cm^2 for 7 days in a chronic constrictive injury model in rats and demonstrated that the use of LLLT could stimulate regeneration, decrease inflammation, and accelerate functional recovery through immunomodulation. Compared to injured, non-treated animals, LLLT irradiation demonstrated a significant reduction in the accumulation of hypoxia-inducible factor (HIF)-1α, down-regulation of pro-inflammatory cytokines TNF-α and IL-1β, and increased expression of VEGF and NGF. Mirsky et al. [91] demonstrated an increase in angiogenesis and endothelial cell proliferation in infarcted rat heart and chick chorioallantoic membrance following irradiation with 804 nm GaAs diode laser.

4.11 Laser acupuncture

Laser acupuncture is defined as the stimulation of acupuncture points using low intensity, non-thermal laser radiation. While it is apparent that LLLT can have distinct biological effects by itself, the use of LLLT at specific anatomical sites may provide additional utility especially since the precise biological mediators are unclear. A double-blind clinical study by Ceccherelli et al. [92] using a pulsed infrared beam applied to the four most painful muscular trigger points and five bilateral homometameric acupuncture points in patients with cervical myofascial pain, found statistically significant pain attenuation in the treatment group. Similarly, Kreczi and Klingler [93] reported statistically significant reduction in pain levels following laser treatment in 21 patients with radicular and pseudoradicular pain syndromes. A large clinical trial conducted with 610 cases by Zhou [94] using a 2.8–6 mW HeNe laser for acupuncture anesthesia for minor operations in the oral maxillofacial region observed satisfactory analgesic effects. However, there are some studies that have reported little, if any, improvements with LLLT on acupuncture trigger points. A study by Waylonis et al. [95] found no statistical difference between the treatment group and placebo groups of 62 patients with chronic myofascial pain using low output HeNe laser therapy. Similarly Haker and Lundeberg [96] conducted a double-blind study on 49 patients suffering from lateral humeral epicondylalgia and applied 940-nm GaAs laser treatment to acupuncture points. They found no statistical difference between the treatment and placebo group. In another study, Lundeberg et al. [97] found no significant changes in evoked

sensory potential of radial nerve and the subcutaneous temperatures in the tissue surrounding the treated radial nerve than placebo in treating cases with tennis elbow. While these contrasting results highlight the importance of dose and methodologies in various studies, the demonstration of a direct ability of LLLT to modulate evoked action potential in dorsal root ganglion neurons establishes distinct physiological (e.g., analgesic) effects [98, 99]. Besides pain, laser acupuncture has also been used to treat postoperative emesis [100], nocturnal enuresis [101], visceral postmenopausal obesity [102] and headaches [103]. Laser acupuncture is rapidly gaining popularity and there are equivocal studies on its clinical applications.

5 Summary and conclusions

Taken together, the results of these experiments demonstrate the potential for LLLT to be used to treat inflammatory conditions. While the exact mechanism of LLLT in modulating pain and inflammation is not fully understood, there is a growing body of evidence supporting the beneficial effects of LLLT from both clinical and basic research studies. Specifically LLLT has been shown to implicate key players in the PII axis such as ROS, ATP, TGF-β, IL-1β, COX, PGE-2 and NF-κB among others. As evident from the cited literature, LLLT appears to have potent clinical efficacy in all of diseased states that have a significant component of PII axis in their etiopathology. Nonetheless, there are also reports of inefficacious use of LLLT. The LLLT dose specifically fluence (J/cm^2), irradiance (W/cm^2) and time along with the biological context and operative mechanisms are key determinants of clinical efficacy [2, 77, 104]. The use of standardized instrument parameters for LLLT is also a key ingredient for clinical success [105, 106]. Along with well-designed clinical studies, the continued exploration of molecular mechanisms will be essential in promoting the progression of LLLT from a mere adjuvant modality to mainstream medicine.

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