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THESIS APPROVAL PAGE FOR MASTER OF SCIENCE IN ORAL BIOLOGY

Title of Thesis: Influence of neodymium-doped yttrium aluminum garnet laser exposure time on cytokine secretion in lipopolysaccharide-challenged rat peripheral blood mononuclear cells

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August 8, 2021

THESIS/MANUSCRIPT APPROVED: DATE:

JOHNSON.THOMAS.MICHAEL.1073956591 Digitally signed by JOHNSON.THOMAS.MICHAEL.1073956591 Date: 2021.08.08 09:44:26 -04'00' 20210808

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Influence of neodymium-doped yttrium aluminum garnet laser exposure time on cytokine secretion in lipopolysaccharide-challenged rat peripheral blood mononuclear cells

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Thesis submitted to the Faculty of the
Army Postgraduate Dental School
Postgraduate Dental College
Uniformed Services University of the Health Sciences
In partial fulfillment of the requirements for the degree of
Master of Science 2022

ABSTRACT

Objective: The objective of this study was to assess the influence of neodymium-doped yttrium aluminum garnet (Nd:YAG) laser exposure time on proinflammatory cytokine/chemokine concentrations in lipopolysaccharide-stimulated rat peripheral blood mononuclear cells (PBMCs).

Materials and Methods: Rat PBMCs were cultured, then stimulated using various lipopolysaccharide (LPS) concentrations—0, 10, 100, or 1000 ng/ml. Cultures at each LPS stimulation level received Nd:YAG laser irradiation (3 or 5 W) over one of four exposure times—0 (control), 30, 45, or 60 s. Pulse duration and repetition rate remained constant at 100 μs and 20 Hz, respectively. We completed magnetic microsphere immunoassays to compare concentrations of 27 inflammatory mediators in laser versus control groups.

Results: In control PBMC cultures, LPS stimulation increased secretion of cytokines and chemokines in a dose-dependent manner. In laser-irradiated cultures, we observed statistically significant reductions in concentrations of four proinflammatory cytokines/chemokines—tumor necrosis factor-α (TNF-α), macrophage inflammatory protein-1α (MIP-1α), macrophage inflammatory protein-2 (MIP-2), and interferon gamma-induced protein-10 (IP-10)—following laser exposures ranging from 30 to 60 s. The effect appeared more pronounced at longer exposure times (45 and 60 s). The lower power setting (3 W) resulted in greater variance in recorded cytokine levels.

Conclusions: Over a range of exposure times, Nd:YAG laser energy produced statistically significant reduction in secretion of 4 of 27 evaluated inflammatory mediators (15%), and the effect was more pronounced with longer exposures (45 and 60 s). Under the experimental conditions described, our findings suggest that Nd:YAG laser irradiation may affect secretion of cytokines and chemokines narrowly rather than broadly, producing statistically significant reductions in concentrations of a small subset of inflammatory mediators. Modifying irradiation parameters may alter the observed anti-inflammatory profile. Future studies should extend our experiments across a range of laser irradiation parameters and cell types.

KEYWORDS: Lasers; leukocytes, mononuclear; inflammation; cytokines; low-level light therapy; lipopolysaccharide

INTRODUCTION

The concept of utilizing light for medical purposes originated long ago. Ancient Egyptian, Greek, Roman, and Arab physicians utilized sunlight to treat a variety of human ailments, beginning as early as 1400 B.C.[1] However, modern phototherapy began to take form around the turn of the twentieth century. In 1903, Niels Ryberg Finsen received a Nobel Prize for his work demonstrating bactericidal and tissue-stimulating effects of concentrated light.[2] In 1960, Theodore Maiman introduced the first laser,[3] and by the end of the same decade, Dr. Endre Mester demonstrated enhancement of a biologic process—hair growth—in mice irradiated with a ruby laser.[4] Since then, researchers have associated laser irradiation with pain relief,[5-11] enhanced wound healing,[5,13-17] bone[18-21] and nerve regeneration,[22-26] and reduced inflammation.[5,27-38]

After Mester's early experiments, investigators quickly realized that progressively large laser exposures or "doses" did not always result in concomitant increases in the recorded biologic outcome—more was not necessarily better.[6] In fact, laser irradiation may produce stimulatory or inhibitory responses, depending upon the applied laser parameters.[2] Of principal importance is the wavelength of the laser light, which determines the absorption profile within tissue.[39] Fluence (J/cm^2) and irradiance (W/cm^2) are also major determinants of the biologic response.[40] Other relevant and interrelated parameters include average power, peak power, pulse duration, pulse repetition rate, exposure time, spot size, target cell type, pulse energy, total energy applied, number of laser applications, and interval between laser applications.[41,42]

Mester referred to the effect he observed as "laser biostimulation,"[4] and low-level laser/light therapy (LLLT) is another commonly used term for the phenomenon.[2] In 2014, the North American Association for Photobiomodulation Therapy (NAALT) and the World Association for Laser Therapy (WALT) jointly affirmed "photobiomodulation" (PBM) as the most appropriate term. WALT defines PBM as induction of nonthermal photophysical and photochemical events in target cells or tissues, leading to physiological changes at various biologic scales.[43] Produced through nonthermal mechanisms, PBM responses are distinguishable from effects that result from simply heating tissue.[2] Typical fluence (energy density) values used in PBM range from 1 through 20 J/cm^2.[40] Above this threshold, thermal effects may predominate or partially account for the observed responses.

Modulation of the inflammatory response is one of the most consistent observations in PBM research, and this effect may account for many of the clinical benefits associated with laser therapy.[28] Various authors have reported reduction in proinflammatory cytokines such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α using infrared lasers.[37,38,44] Infrared laser irradiation also appears to decrease cyclooxygenase (COX)-2 mRNA levels and prostaglandin synthesis in some cell types.[33] Given the number of laser parameters potentially influencing cell/tissue effects and the complexity of the immune response, additional research is necessary to clarify underlying PBM mechanisms and optimize therapeutic protocols.

OBJECTIVE

We aimed to evaluate the influence of a specific laser parameter (exposure time) on cytokine/chemokine secretion in stimulated peripheral blood mononuclear cells (PBMCs) irradiated with a neodymium-doped yttrium aluminum garnet (Nd:YAG) laser.

MATERIALS AND METHODS

Target Cells

Rat PBMCs (IQ Biosciences, Berkeley, California) were cultured and exposed to one of four lipopolysaccharide (LPS) concentrations—0, 10, 100, or 1000 ng/ml. We then transferred the cells to 96-well plates (2.0×10^4 cells per well).

Laser Irradiation Parameters

We used an Nd:YAG laser (1064 nm, Lightwalker AT, Fotona, Dallas, Texas) to irradiate cultures in laser treatment groups. A 300-micron optical fiber directed the laser beam perpendicularly to the plated cells from a distance of 1.8 mm, and a standardized support reliably reproduced the laser position. Cultures at each LPS stimulation level received Nd:YAG laser irradiation (3 or 5 W average power) at one of four exposure times—0 (control), 30, 45, or 60 s (Figure 1). Pulse duration and repetition rate remained constant at 100 μs and 20 Hz, respectively. After irradiation, we incubated the cells for one hour at 37˚ C prior to analysis.

Evaluation of Cytokine/Chemokine Concentrations

We utilized a magnetic microsphere immunoassay (MAGPIX System, Luminex, Austin, Texas) to simultaneously analyze 27 cytokine/chemokine levels in each culture (Table 1). The immunoassay kit included color-coded magnetic microspheres tagged with fluorescent dyes covalently coupled with antibodies specific to cytokines of interest. After we subjected the microspheres to a culture, we introduced a reporter molecule labeled with a specific fluorescent dye. A light emitting diode (LED) excited the dyes, and a CCD camera detected the fluorescence from each microsphere. Median fluorescence intensities (MFIs) provided the basis for the sample analysis. The analysis software (MAGPIX System, Luminex) processed the images and determined cytokine/chemokine concentrations in picograms per milliliter (pg/ml) using standard curves.

Statistical Analysis

We performed each experiment (LPS concentration and laser treatment combination) in duplicate wells per condition and in two independent experiments. Data are presented as means ± SEM. Mean cytokine concentrations for treatment and control groups were compared using Student's t-tests. Differences were accepted as significant at the $p<0.05$ level.

RESULTS

Four of 27 inflammatory mediators analyzed (15%) exhibited statistically significant reductions in concentration in the laser-irradiated compared with control cultures. As expected, we observed dose-dependent increases in cytokine/chemokine concentrations in the

control groups (Figure 2). We detected some statistically significant differences at each laser exposure (30, 45, and 60 s). However the anti-inflammatory effect was more pronounced at the higher exposure times (45 and 60 s) (Figure 3). Cytokine/chemokine concentrations recorded in cultures exposed to the lower power setting (3 W, 60 s exposure time) were not statistically different than corresponding concentrations in control cultures. Additionally, in the cultures receiving 3 W power exposure, cytokine/chemokine concentrations exhibited greater variation (standard deviation) in repeated experiments. Some cytokines exhibited a trend for increased concentrations following exposure to laser power of 3 W, although this trend did not reach statistical significance.

DISCUSSION

Our objective was to evaluate the effect of Nd:YAG laser exposure time on secretion of 27 inflammatory mediators in LPS-stimulated rat PBMCs. Although some statistically significant reductions in cytokine concentrations were noted at each evaluated exposure time, the longer exposures (45 s and 60 s) did produce more pronounced anti-inflammatory effects. Because general reduction in inflammation is a consistent finding in PBM studies,[28] an expectation of extensive laser influence on a large proportion of the evaluated proteins may have been reasonable. On the contrary, we observed statistically significant reductions in only four proinflammatory cytokines/chemokines. It is possible that the power levels we applied exceeded ideal for producing anti-inflammatory effects. Notably, our experiments were incapable of establishing optimal parameters to minimize proinflammatory cytokine levels. Since numerous PBM outcomes of interest exhibit biphasic responses,[40] it is possible that a

different fluence value (J/cm^2) may have affected the evaluated inflammatory mediators more broadly and to a greater degree.

Most of our experiments utilized an average laser power of 5 W. We selected this power level based on the findings of a previous study in our lab. Utilizing an experimental design similar to that of the present study, Bunting and colleagues assessed cytokine concentrations after irradiating cultures for 30 s with one of four power values—0 (control), 5, 7.5, or 10 W.[45] Bunting found in cultures receiving 7.5- and 10-W laser exposures, cytokine concentrations were reduced below detection limits.[45] The maximum exposure time used in the present investigation (60 s) was twice the exposure time Bunting applied.[45] Consistent with Bunting's findings, we noted a less pronounced anti-inflammatory effect, as well as higher standard deviation, in the group receiving the lower power value (3 W). Additionally, our observation of a trend for increased concentrations of some cytokines in the 3 W group may suggest a biphasic response related to laser power. Our findings, in the context of Bunting's work, indicate that higher power (7.5 to 10 W) over a shorter exposure period (30 s) or moderate power (5 W) over a longer period (45 to 60 s) may produce superior suppression of some proinflammatory cytokines.

CONCLUSIONS

Under the experimental conditions described, our findings suggest that Nd:YAG laser irradiation may affect secretion of cytokines and chemokines narrowly rather than broadly, producing statistically significant reductions in concentrations of a small subset of the evaluated inflammatory mediators. A more pronounced anti-inflammatory effect occurred with longer

laser exposures (45 and 60 s). Modifying irradiation parameters may alter the observed anti-inflammatory profile. Future studies should extend our experiments across a range of laser irradiation parameters and cell types.

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FIGURES

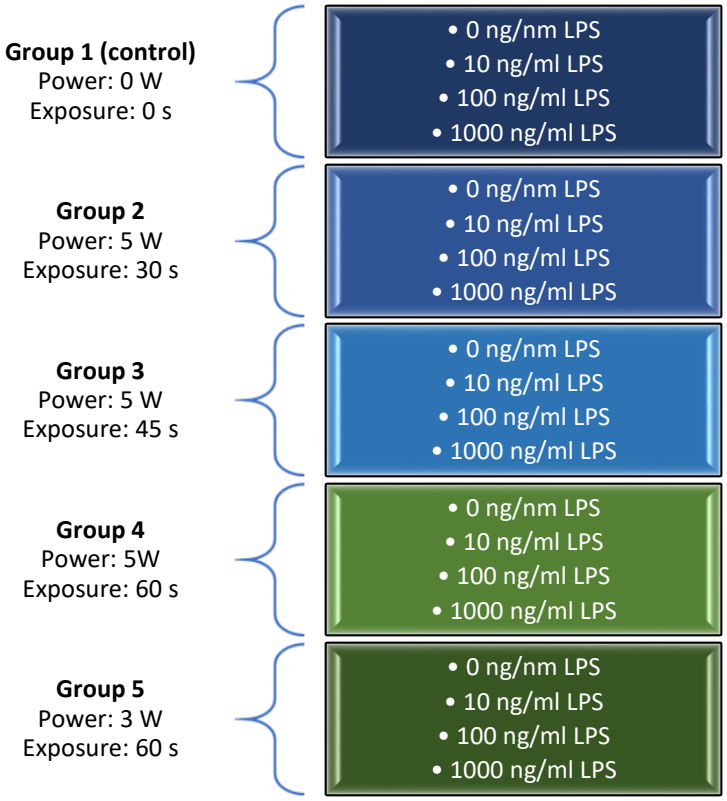

Figure 1. Experimental design. In all groups, we held pulse duration and repetition rate constant at 100 s and 20 Hz, respectively. We completed each experiment (LPS concentration and laser exposure combination) in duplicate.

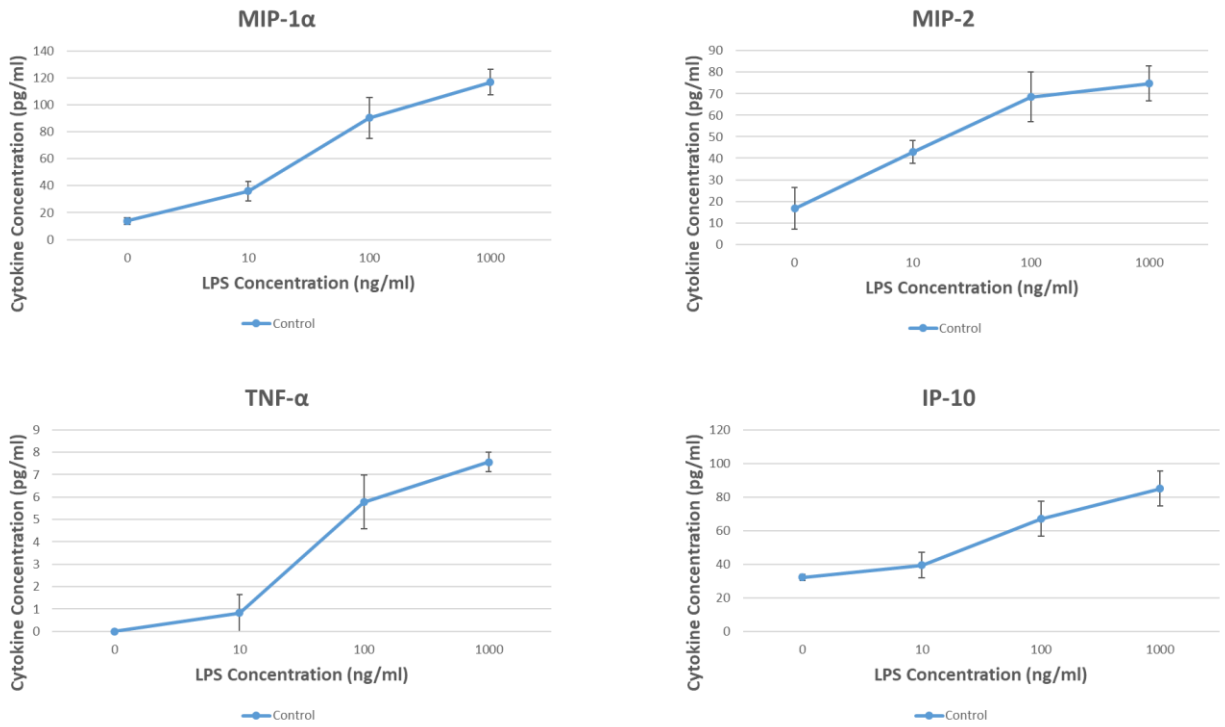

Figure 2. Control cultures for inflammatory mediators exhibiting statistically significant concentration reductions. In control cultures, cytokine/chemokine and lipopolysaccharide (LPS) concentrations correlated appropriately.

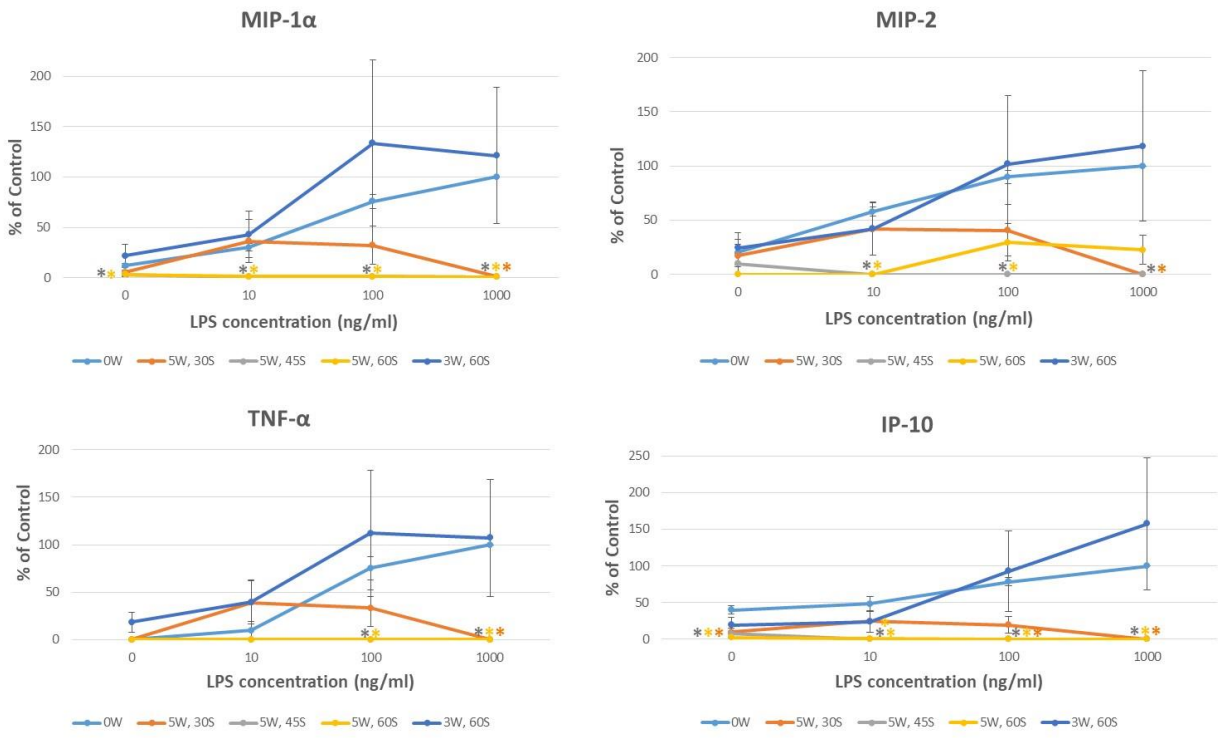

Figure 3. Normalized mean concentrations of cytokines/chemokines exhibiting statistically significant differences compared with controls.

TABLES

Table 1. Inflammatory mediators evaluated by magnetic microsphere immunoassay

Tumor necrosis factor-α (TNF-α)
Macrophage inflammatory protein-1α (MIP-1α)
Macrophage inflammatory protein-2 (MIP-2)
Interferon gamma-induced protein 10 (IP-10)
Granulocyte colony-stimulating factor (G-CSF)
Eotaxin
Granulocyte-macrophage colony-stimulating factor (GM-CSF)
Interleukin-1α (IL-1α)
Leptin
Interleukin-4 (IL-4)
Interleukin-1 β (IL1- β)
Interleukin-2 (IL-2)
Interleukin-6 (IL-6)
Epidermal growth factor (EGF)
Interleukin-13 (IL-13)
Interleukin-10 (IL-10)
Interleukin-12p70 (IL-12p70)
Interferon (IFN)
Interleukin-5 (IL-5)
Interleukin-17α (IL-17α)
Interleukin-18 (IL-18)
Monocyte chemoattractant protein-1 (MCP-1)
Growth-regulated oncogene/keratinocyte chemoattractant (GRO/KC)
Vascular epithelial growth factor (VEGF)
Fractalkine
Liposaccharide-Induced CXC chemokine (LIX)