from 0 to 20 minutes at 5 minute intervals. One solution each of all live (positive) or all dead (negative) non-treated controls were included for comparison. The solutions were treated once in microcentrifuge tubes with shockwaves generated from laser ablation of titanium on polyimide substrates. Filters at 561 nm and 488 nm were used to analyze the cells under a SORP BD LSRII Analytic Flow Cytometer.
Results: The solution with non-treated live cells showed little absorption of PI, but all treated solutions showed stronger absorbance levels. 20 minutes after LGS treatment, the cells still demonstrated persistent uptake of PI. The dead cells showed the highest level of PI absorbance.
Conclusion: The results suggest that LGS may have a direct permeabilization effect on the bacterial cells. The low PI values for the non-treated live cells and higher PI values for the treated cells support this. Future studies need to assess the impact of multiple treatments to the bacteria. This data confirms that LGS can be a potentially useful adjunct to the treatment of bacterial biofilms in chronic wound and soft tissue infections.

BASIC SCIENCE AND TRANSLATIONAL RESEARCH: *PHOTOBIOMODULATION*

EFFECT OF PHOTOBIOMODULATION ON AN EXPERIMENTAL MODEL OF CHRONIC ASTHMA: PARTICIPATION OF LEUKOTRIENES

Auriléia A. Brito, Karina Brajato, Veridiana Delle, Clariana Rodrigues Soares, Stephanie S. Soares, Ana Paula L. Oliveira
Universidade Nove de Julho, São Paulo, Brazil

Background: Studies leukotriene receptor antagonists, such as montelukast (MK) has contributed at different levels for the treatment of asthma. Our studies indicate that LLL therapy is effective in reducing allergic inflammation and pulmonary remodeling in an experimental model of lung diseases. Thus, the present study aimed to evaluate the effects of LLL therapy on an experimental model of ovalbumin-induced chronic pulmonary allergic inflammation (OVA).
Study Design/Materials and Method: BALB/C mice were divided into four groups: Basal, LLL, OVA, OVA + LLL. Chronic lung allergic inflammation was induced by ovalbumin sc (OVA) immunization, mixed with alum (days 0 and 14) and oral tracheal challenge with OVA (three days/week for five weeks). The OVA + LLL group was irradiated (diode laser, 660 nm, 30 mW, and 3J). Twenty four hours after the last treatment, the animals were anesthetized, tracheotomized, cannulated and the bronchoalveolar lavage was collected and analyzed (total and differential cell counts as well as cytokine levels in the bronchoalveolar lavage through the elisa technique).
Results: Both treatments reduced the total number of cells and eosinophils in bronchoalveolar lavage (BAL) ($p < 0.001$). However, in the simultaneous treatments the reduction was more significant ($p < 0.01$). There was a significant reduction ($p < 0.05$). In IL-5 levels of all treated groups, IL-13 levels in the treated groups were significantly reduced ($p < 0.001$). Regarding leukotrienes, we noticed a significant decrease ($p < 0.05$) in LTB4 levels in the OVA + LLL group in relation to the OVA group. There was a reduction in the deposition of collagen and mucus fibers in the airways in the treated group ($p < 0.001$), in the evaluation of pulmonary mechanics, a significant decrease ($p < 0.001$) was observed in the treated group, in all situations evaluated.
Conclusion: Thus, these results indicate that is effective in reducing allergic inflammation, remodeling and pulmonary elastance. These effects appear to be mediated by modulation of the LLL in the secretion of the leukotrienes.

EFFICACY OF PHOTOBIOMODULATION THERAPY IN MITIGATING SKIN RADIATION DAMAGE

Rodrigo Crespo Mosca, Sofia Nascimento Santos, Gesse Eduardo Calvo Nogueira, Daisa Lima Pereira, Francielli Campos Costa, Praveen Arany, Carlos Alberto Zeituni
Nuclear and Energy Research Institute, Radiation Technology Center, São Paulo, Brazil; Oral Biology & Biomedical Engineering School of Dental Medicine, Engineering & Applied Sciences, University at Buffalo, Buffalo, NY

Background: The use of sophisticated radiation dose delivery and fractionation has significantly improved cancer care. One of these involves localized, sustained ionizing dose delivery termed brachytherapy. Despite it therapeutic efficacy, specific side effects of brachytherapy include localized skin damage and breakdown for which only palliative treatments are currently available. The use of low dose biophotonics treatments to promote tissue healing is termed photobiomodulation (PBM) therapy. The aim of this study was to evaluate efficacy and molecular pathways of PBM therapy using two common wavelengths, red and near-infrared (NIR) to treat radiation wounds in athymic mice subjected to brachytherapy (sustained ionizing radiation from 125I seeds).
Study Design/Materials and Method: A pilot study was performed with thirty-six athymic mice were accomplished for 60 days and divided into six groups: Surgical Control Group (No radiation and no PBM treatments); Radiation Control Group (125I seed 0.4252 mCi, no PBM); NIR-PBM Control Group (NIR PBM alone, LED at $\lambda = 880$ nm); Red-PBM Control Group (Red PBM alone LED at $\lambda = 660$ nm); Radiation- NIR PBM Group; Radiation-Red PBM Group. Following 21 days, radiation-induced wounds are evident. PBM treatments (both wavelengths with output power 40 mW for 20 s, fluence 20 J/cm^2 on top of implantation site) were performed every week up to 60 days. Wounds were evaluated every 7 days digital imaging, Laser Doppler Flowmetry (LDF) and tissue temperature with a thermographic camera. We also performed μPET-CT imaging using radioactive fluorodeoxyglucose (18F-FDG) at 51 and 81 days post-implantation. Animals were sacrifices progressively at each time point to correlate clinical observations with imaging and molecular tissue analyses. Tissues were collected to analyze molecular pathways correlating with inflammation, immune response, wound healing and angiogenesis using mRNA (qRT-PCR) and protein expression (immunostaining).
Results: Both PBM treated groups demonstrated significant ($p < 0.05$) improvements in skin radiation wound healing as compared to radiation group. Distinct improvements in clinical wound size and closure, improved tissue perfusion and reduced inflammation as evidenced by decreased wound thermal images. These wounds were also noted to have significant differences in the cytokine profiles (TGF-β, VEGF and PDGF) correlating with better healing responses. Radiation damage reduces brown fat composition that can potentially contribute to additional radiation-associated morbidities. The μPET-CT imaging noted significant preservation of brown fat composition in PBM-treated

radiation alone groups. Further validation of these pathways is ongoing.
Conclusion: Within the parameters of this study, PBM treatments demonstrated improved healing in radiation wounds due to ionizing radiation from 125I seeds. Ongoing work is examining the precise molecular pathways contributing to these therapeutic benefits. It is hoped this study will enable further development of this innovative therapy for managing side-effects from radiation treatments.

ELECTROMYOGRAPHIC EVALUATION OF MOTOR RESPONSE TO PHOTOBIOMODULATION FOR THE TREATMENT IN PATIENTS WITH SPINAL CORD INJURY

Andréa O. Gomes
Universidade Nove de Julho, São Paulo, Brazil

Background: Traumatic spinal cord injury can range from mild medullary concussion to transient dormancy and permanent quadriplegia. The most common sites of this lesion are at the level of the cervical vertebrae, C5, C6 and C7 and at the level of the thoracic and lumbar vertebrae, T12 and L13. The literature is unclear as to an effective therapy to improve the quality of life of patients with spinal cord injury. Photobiomodulation is a promising resource in musculoskeletal injuries, so the role of this study is to assess the potential of phototherapy in patients with sensorimotor deficit.
Study Design/Materials and Method: This study involves 25 patients with a diagnosis of spinal cord injury recruited at the Physiotherapy Clinic of the University of Nove de Julho (UNINOVE, Brazil), who underwent 12 sessions of phototherapy with electromyographic evaluation at the beginning and at the end of the sessions. The irradiation was administered to the site of injury transcutaneously using a *Quantum* diode laser (Ecco Fibers and Devices, Brazil) with wavelength of 808 nm, aperture diameter 0.18 cm, Irradiance at aperture 4.72 W/cm, Number of 5 points irradiated, area irradiated of the 0.0254 cm and frequency of treatment sessions of the twelve sessions were performed on three per week for four weeks. The EMG signals were captured using a four-channel acquisition system (EMG 432C, EMG System do Brazil Ltda.) consisting of a signal conditioning module, bipolar active electrodes, analog band pass filter from 20 to 500 Hz and common mode rejection ratio of 120 dB. The sampling frequency was 2 kHz, scanned using analog/digital (A/D) conversion board with 16 bits of resolution.
Results: In the phototherapy group, median frequency values of the brachial biceps and femoral quadriceps muscles were higher at rest and during isotonic contraction 30 days after photobiomodulation ($p = 0.0258$). No significant results were found regarding to the rest and isotonic conditions in the pre-photobiomodulation period ($p = 0.950$) or immediately following photobiomodulation ($p = 0.262$).
Conclusion: The data provide evidence that phototherapy improves motor response in individuals with spinal cord injury through the difference before and after treatment with phototherapy.

INCREASED MITOCHONDRIAL METABOLISM BY TWO DIFFERENT WAVELENGTHS DID NOT RESULT IN ALTERED FIBROBLASTIC PROLIFERATION *IN VITRO*

Juanita J. Anders, Xingjia Wu, Ilko K. Ilev
Uniformed Services University of the Health Sciences, Bethesda, MD; Food and Drug Administration, Silver Spring, MD

Background: Previous photobiomodulation (PBM) studies on *in vitro* fibroblasts reported increased cellular proliferation. Typically these culture models had decreased serum levels in the media so that growth conditions were not optimal. The purpose of this study was to determine if combinations of irradiance (mW/cm^2) and time resulting in different fluences (J/cm^2), for different wavelengths, that were effective in altering mitochondrial metabolism as measured by the MTS assay also resulted in cellular proliferation using adult human dermal fibroblasts (HDF) grown in high glucose medium.
Study Design/Materials and Method: The laser wavelengths used were: 810 nm and 980 nm. A precision light delivery device which included an electronic shutter was used. Adult HDF were cultured in chamber slides (10,000 cells/well) with defined medium (control) or the control medium with high glucose (180 mM). Cells in high glucose medium were treated with the following laser parameters which we previously identified as significantly altering mitochondrial metabolism: 50, 100, 200, and 300 mW/cm^2 and 0.05, 0.2, 1, 5, 20 J/cm^2. All settings were replicated four times. Twenty-four hours after laser treatment, cells were washed and frozen at −80 °C overnight. The CyQuant assay was applied to measure the DNA-based proliferation. Statistical analysis was done using one way ANOVA with Tukey multiple comparison test.
Results: Although all the laser parameters used had significantly increased mitochondrial metabolism in the HDF, this effect did not result in increased cellular proliferation in this high glucose *in vitro* model.
Conclusion: It is commonly stated in the literature that PBM results in improved cell survival, increased proliferation and migration, and new protein synthesis implying that all these results occur after PBM therapy. This data demonstrates that the secondary effects that occur after light absorption and increased mitochondrial activity depends on the state of the cell and are dictated by the physiological needs of the cell.

LOW LEVEL LASER THERAPY INHIBITS NEUROPATHIC PAIN AND RESTORES THE MORPHOLOGICAL PATTERN OF THE SCIATIC NERVE OF DIABETIC MICE

Victória da Silva Oliveira
University of São Paulo, São Paulo, Brazil

Background: Diabetic peripheral neuropathy (DPN) is one of the most common complications caused by diabetes mellitus and the development of chronic pain is the most prevalent symptom. Conventional treatments for DPN are still unsatisfactory, leading to the search for new therapies. Low level laser therapy (LLLt) arises as a new alternative target, through its analgesic, anti-inflammatory and biomodulators effects inducing a significant improvement of disabilities observed on DPN. Herein we evaluated the therapeutic potential of LLLt in a model of diabetic neuropathy induced by streptozotocin (STZ) in mice, as well as possible mechanisms involved in its effect.
Study Design/Materials and Method: Male, C57BL/6 mice (20–26 g; 8 weeks old − CEUA-ICB 22/2014) were used thought this study. Animals received a single injection of STZ (225 mg/kg i.p.) and, after 14 days, mechanical hypersensitivity was confirmed by von Frey filaments. LLL was applied to the left hind paw (1.6 J/cm^2; 30 mW; 15 secs, spot size 0.28 cm^2) for 21 consecutive days. Behavioral testing was repeated after 7, 14 or 21 LLL-sessions. Sciatic nerves were removed at the 21st day for nerve growth factor (NGF) evaluation by ELISA and for morphology evaluation by transmission electronic microscopy. Behavioral tests were analyzed by two-way ANOVA followed by Bonferroni's post-test; NGF quantification was analyzed by nonparametric t test. The significance level considered was $p < 0.05$.