Effect of NASA Light-Emitting Diode Irradiation on Molecular Changes for Wound Healing in Diabetic Mice

HARRY T. WHELAN, M.D.,[1] ELLEN V. BUCHMANN, B.S.,[1] APSARA DHOKALIA, Ph.D.,[2]
MARY P. KANE, B.S.,[1] NOEL T. WHELAN, B.S.,[1] MARGARET T.T. WONG-RILEY, Ph.D.,[3]
JANIS T. EELLS, Ph.D.,[4] LISA J. GOULD, M.D., Ph.D.,[5] RASHA HAMMAMIEH, Ph.D.,[2]
RINA DAS, Ph.D.,[2] and MARTI JETT, Ph.D.[2]

ABSTRACT

Objective: The purpose of this study was to assess the changes in gene expression of near-infrared light therapy in a model of impaired wound healing. *Background Data:* Light-Emitting Diodes (LED), originally developed for NASA plant growth experiments in space, show promise for delivering light deep into tissues of the body to promote wound healing and human tissue growth. In this paper we present the effects of LED treatment on wounds in a genetically diabetic mouse model. *Materials and Methods:* Polyvinyl acetal (PVA) sponges were subcutaneously implanted in the dorsum of BKS.Cg-*m* +/+ *Lepr^db* mice. LED treatments were given once daily, and at the sacrifice day, the sponges, incision line and skin over the sponges were harvested and used for RNA extraction. The RNA was subsequently analyzed by cDNA array. *Results:* Our studies have revealed certain tissue regenerating genes that were significantly upregulated upon LED treatment when compared to the untreated sample. Integrins, laminin, gap junction proteins, and kinesin superfamily motor proteins are some of the genes involved during regeneration process. These are some of the genes that were identified upon gene array experiments with RNA isolated from sponges from the wound site in mouse with LED treatment. *Conclusion:* We believe that the use of NASA light-emitting diodes (LED) for light therapy will greatly enhance the natural wound healing process, and more quickly return the patient to a pre-injury/illness level of activity. This work is supported and managed through the Defense Advanced Research Projects Agency (DARPA) and NASA Marshall Space Flight Center–SBIR Program.

INTRODUCTION

THE NEED TO CARE for a population with chronic wounds is a growing challenge that requires innovative approaches. One approach that specifically addresses the identified pathophysiological processes involved in wound healing is light therapy. We believe that the use of NASA light-emitting diodes (LED) for light therapy will greatly enhance the natural wound healing process. This will save valuable time and resources for both patients and health care facilities. Furthermore, improved wound healing will reduce the risk of infection for the patient, decrease the amount of costly dressings required, and more quickly return the patient to a pre-injury/illness level of activity.

Laser light has been widely acclaimed to speed wound healing of ischemic, hypoxic, and infected wounds.[1] Lasers provide low energy stimulation of tissues that results in increased cellular activity during wound healing.[2,3] These activities include collagen production and angiogenesis.[4]

Wound healing has three phases: first a substrate is laid down, then cells proliferate, and finally there is remodeling of tissue. The data published so far suggests that laser biostimulation produces its primary effect during the cell proliferation phase of the wound healing process. It has been demonstrated that mitochondria are receptive to monochromatic near-infrared light and that laser light likely increases respiratory metabolism of certain cells.[2,3,5] Processes such as fibroblast proliferation, attachment and synthesis of collagen and pro-

[1]Department of Neurology, Medical College of Wisconsin, Milwaukee, Wisconsin.
[2]Department of Molecular Pathology, Walter Reed Army Institute of Research, Silver Spring, Maryland.
[3]Department of Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, Wisconsin.
[4]Department of Pharmacology, Medical College of Wisconsin, Milwaukee, Wisconsin.
[5]Department of Plastic Surgery, University of Texas Medical Branch, Galveston, Texas.

collagen, growth factor production [including keratinocyte growth factor (KGF), transforming growth factor (TGF) and platelet-derived growth factor (PDGF)], macrophage stimulation, lymphocyte stimulation[6] and greater rate of extracellular matrix production have been reported with laser light treatment.[7–14] Animal studies on the enhanced wound healing effect of laser light of low power density have been performed in toads, mice, rats, guinea pigs, and swine.[15,16] Human studies with laser light have demonstrated greater amounts of epithelialization for wound closure and stimulation of skin graft healing.[1,9] An excellent review of recent human experience with near-infrared light therapy for wound healing was published by Conlan et al. in 1996.

Lasers, however, have some inherent characteristics which make there use in a clinical setting problematic, including limitations in wavelength capabilities and beam width. The combined wavelengths of the light for optimal wound healing cannot be efficiently produced, the size of wounds which may be treated is limited (due to laser production of a beam of light; a fact inconsistent with treating large areas), heat production from the laser light itself can actually damage tissue, and the pin-point beam of laser light can damage the eye. NASA-developed LEDs offer an effective alternative to lasers. These diodes can be configured to produce multiple wavelengths, can be arranged in large, flat arrays (allowing treatment of large wounds), and produce no heat. It is also of importance to note that LED light therapy has been deemed a nonsignificant risk by the FDA; thus, FDA approval for the use of LEDs in humans for light therapy has been obtained.

NASA LEDs stimulate the basic energy processes in the mitochondria (energy compartments) of each cell, particularly when near-infrared light is used to activate the wavelength sensitive constituents inside (chromophores, cytochrome systems). Optimal light wavelengths (proven in prior studies of laser and LED light[2,3,8,11–14,17] to speed wound healing include 680 nm, 730 nm, and 880 nm. These wavelengths can be produced accurately be NASA LEDs, which have a bandwidth of 25nm. The depth of near-infrared light penetration into human tissue has been measured spectroscopically.[2,3,18] Spectra taken from the wrist flexor muscles in the forearm and muscles in the calf of the leg demonstrate that most of the photons at wavelengths of 630–800 nm travel approximately 23 cm through the skin surface (light input) and muscle, exiting at the photon detector. Data collection and cataloging to elucidate the absorption coefficients of the various human tissues is currently underway by this principle investigator.

We have used the cDNA array technology to discover the expression of various genes that are induced upon LED treatment and followed through the entire process of healing trying to identify some of the early mid and late events at the molecular level. Knowledge gained from determining cellular and molecular mechanisms will direct our improvements in noninvasive therapeutic technologies.

MATERIALS AND METHODS

Eighty genetically diabetic mice (BKS.Cg-*m +/+ Lepr*[db]) from Jackson Laboratory (Bar Harbor, ME) were divided into two groups of 40 mice each. The groups included a control and a group treated with a 670-nm LED. The LED treatments were given daily at a fluence of 4 J/cm2 for 14 days.

Animal care and surgery were performed in accordance with an approved protocol by the Animal Resource Center at the Medical College of Wisconsin. The animals were anesthetized through inhalation of Isoflurane. The dorsum of the anesthetized animal was shaved and disinfected with Betadine. Using aseptic technique, two 0.5-cm incisions were made on either side of the spine just inferior to the scapulae. Two individual, subcutaneous pockets were dissected caudally from the incisions. Two PVA sponges (Merocel, Mystic, CT) that measured $3 \times 3 \times 5mm$ were inserted into the pockets (one sponge per pocket). The incisions were closed with 6-0 Ethilon (Ethicon, Somerville, NJ). The animals were caged individually and given food and water ad libitum.

Once per day, the animals were placed in an open-top Plexiglas restrainer, and the 670-nm LED was placed over the animals. The LED treatments were given at a power of 28 mW/cm2 for 2 min and 24 sec to achieve a dose of 4 J/cm2.

Each of the two groups were subdivided into four groups of 10 animals each. On days 2, 4, 7, and 14 of the treatment period, animals were sacrificed and samples were collected for cDNA microarray analysis. The incision line was excised, the sponges were removed, and the skin overlying the sponges was excised. These samples were immediately placed in Trizol reagent (Invitrogen, Carlsbad, CA). The samples from each group were pooled, homogenized using a polytron, and the RNA was precipitated using the manufacturer's instructions. The precipitated RNA was stored on ethanol at −80°C. The samples were shipped on dry ice for microarray analysis.

RNA isolation and microarray

RNA obtained using the Trizol method[19] was further processed for DNAse digestion and purification.

For RNA samples obtained during the study, we used mouse cDNA arrays containing ~1,200 genes. For each cDNA array RNA was labeled with ^{33}P and exposed to a Kodak screen that was scanned using BIORAD Multiflour Scanner FX. The scanned images were then analyzed using ATLAS Image 2.0 software. The data obtained was then analyzed for gene clustering using Cluster and TreeView programs.[20] The unsupervised clustering algorithm was applied to microarray analysis of those samples. More comprehensive analysis of the differential gene expression was carried out using GeneSpring from Silicon Genetics.

RESULTS

Our initial studies with skin wound healing model in mouse have revealed certain patterns of gene expression induced by LED. Tissue regenerating genes were significantly upregulated upon LED treatment when compared to the untreated sample. Integrins, nidogen, laminin, actin, kinesin motor proteins are some of the genes that have been reported to be involved during regeneration process. These are some of the genes that were identified upon gene array experiments with RNA isolated from sponges from the wound site in mouse with and without LED treatment.

A wound healing impaired type 2 diabetic mouse model has been studied. As previously reported, genetically diabetic mice treated with low level laser irradiation demonstrated significantly enhanced wound closure grossly, and improved wound epithelialization, cellular content, granulation tissue formation, collagen deposition, and extensive neovascularization on histological evaluation.[21] In our study, type 2 diabetic mice with excisional skin wounds were treated with LEDs at individual wavelengths of 680 nm, 730 nm, and 880 nm at 4 J/cm^2 and 50 mW/cm^2. LED treatment produced increased healing rates, compared to surgical controls as seen in Figure 1.

A repeated measures analysis was conducted using a General Linear Model with *SqrtArea* as the dependent variable and *Treat* as the independent variable. The interaction effect Day*Treat is significant ($p = 0.0095$), indicating that there is a significant difference between treatments on some days. This test is of primary interest in this situation, because it shows that the treatments are effective for some part of the treatment period. This analysis was carried out using the SAS statistical software package, published by The SAS Institute, Inc.

Gene changes induced by LED can be categorized into two major groups, gene that were upregulated (Fig. 2A) in both time periods and genes that were downregulated in both time periods (Fig. 2B). LED stimulated genes coding for improved tissue regeneration and basement membrane repair.

Basement membrane and tissue regenerating genes were significantly upregulated in LED versus untreated control.

Downregulated genes were clustered using the hierarchical cluster, and genes that were downregulated in both time periods were selected (Fig. 2B).

The basement membrane consists of a supramolecular network of collagen type IV, laminin (LN), nidogen, and associated proteoglycans. Increased expression of basement membrane components during sequential phases of wound angiogenesis and healing was repeatedly observed upon LED treatment.

Few selected genes out of approximately 306 genes that were significantly altered have been compared for their expression levels at the two time points studied after LED treatment (Fig. 3). These preliminary results are based on gene array experiments however they wound need to be confirmed by other quantitative techniques.

Laminin, nidogen, myosin were among the many genes that are part of the basement membrane were upregulated at both time points by LED. Genes from the kinesin superfamily proteins that are involved in regeneration were also altered. Kinesin superfamily motor proteins are involved during regeneration.

Semaphorins/collapsins, a family of genes with a semaphorin domain conserved from insects through to mammals, have been shown to be involved in axon guidance during neuronal development in addition to the axon repellent function of semaphorin D. Semaphorins are involved in axon guidance during neuronal development in addition to the axon repellent function of semaphorin H.

Galectin-7 is a beta-galactoside binding protein of the lectin family, specifically expressed in stratified epithelia and notably in epidermis. Its production coincides with the degree of stratification of the epithelia. It can be considered as a marker of all subtypes of keratinocytes. This gene was upregulated at day 2 and continued to be elevated after 14 days of LED treatment (Fig. 3).

Fibroblast growth factor 7 and 12 were also upregulated by 2 days upon LED treatment in the sponge site of diabetic mice. These are growth factors known to be involved in the regeneration process (Fig. 3). Genes for TGF-beta 1 and thrombospondin 1 (TSP-1) were however upregulated by 14 days of LED treatment.

Calcium regulated genes, Calpactins, were also altered by LED treatment. Calpactins are a family of related Ca^{2+}-regulated cytoskeletal proteins. The light chain is a member of the S100 family known to be associated with cell differentiation, malignant transformation, and S-phase of cell cycle.

Genes such as receptor for cytokines, cytokines such as interleukin-1, IL-10, macrophage inflammatory protein-2, and proapoptosis associated genes are a few that were downregulated at both time points studied (Fig. 4).

Expression levels of each of these genes have been observed only by several gene array experiments. However each of these selected genes will need to be confirmed by other quantitative techniques such as real-time PCR (RT-PCR).

DISCUSSION

The biochemical mechanism by which LED enhances the process of wound healing is not known. The current theory is that the infrared light is absorbed by some photoreceptors, which then trigger a cascade of reactions in a cell. The major biological photoacceptors in the near-infrared range have been determined to be hemoglobin, myoglobin, and cytochrome oxidase. LED treatment effectively energized the cells by stimulating their cytochrome oxidase[12,13] and triggered a cascade of cellular and molecular events that have significant biological benefits.

Using the gene array technology, we observed a variety of gene families such as basement membrane components to be upregulated by LED when compared to the untreated controls.
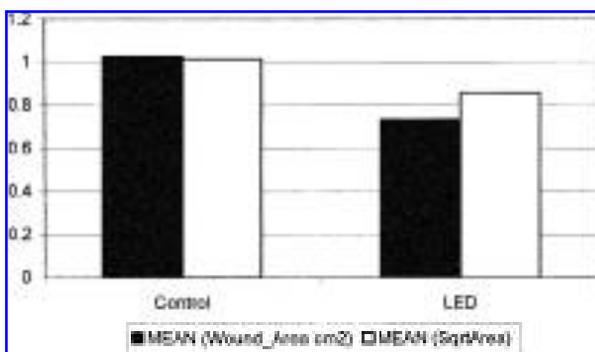
FIG. 1. Type 2 diabetic mice with excisional skin wounds treated with combined LED wavelengths, 4 J/cm^2, 50 mW/cm^2. The square root of wound area is used in the dependent variable in the analysis. This transformation was needed to correct for non-constant error in the General Linear Model. SqrtArea could be interpreted as being proportional to the radius of a circular wound. The interaction effect Day*Treat is significant ($p = 0.0095$).

A

B

20.140

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5-hydroxytryptamine serotonin receptor 1e beta
ENDOTHELIAL TRANSCRIPTION FACTOR GATA-2
NKT-1; lymphocyte differentiation antigen CD38
type II cytoskeletal keratin KRT2-1; cytokeratin 1 KRT1; 57-kDa cytokeratin
CD3 antigen
BH3 interacting domain death agonist BID
MEKK5 MAP/ERK kinase kinase 5 ASK1, MAPKKK5, mitogen activated protein kinase kinase 5
epidermal growth factor EGF
STEROID HORMONE RECEPTOR ERBB1 ESTROGEN-RELATED RECEPTOR, ALPHA HER-ALPHA ESTROGEN RECEPTOR-LIKE 1
programmed cell death 1 protein precursor PDCD-1, PD-1
Semaphorin IIIC SEMA3C; semaphorin E precursor SEMA3; SEMB
PERIPHERAL MYELIN PROTEIN 22 PMP-22 CD25 PROTEIN GAS3 MYELIN PROTEIN.
mitochondrial matrix protein P1 precursor; p60 lymphocyte protein; 60-kDa chaperonin; heat shock 60-
CDC42 GTP-binding protein; G25K
TNF receptor-associated factor 3 TRAF3; CD40 receptor-associated factor 1 CRAF1; TRAFAMN
zinc finger protein 47 ZFP47
Myogenin MYOG, myoD1-related protein
5-hydroxytryptamine receptor 1B receptor 5HT1B; serotonin receptor
C-C chemokine receptor; monocyte chemoattractant protein 1 receptor MCP-1RA
transcription factor 8 TCF8; transcription repressor deltaEF1; Zeb1
growth/ differentiation factor 1 GDF-1; TGF- beta family
BIAP3; inhibitor of apoptosis protein 3 X-linked inhibitor of apoptosis protein X-linked IAP IAP hom
homolog of chicken slug zinc finger protein SLUGH
CD3 antigen delta polypeptide
distal-less homeobox protein 5 DLX5
nucleix nsmbl; mskl
probable calcium-binding protein ALG2; PMP41; ALG-257
VITAMIN D3 RECEPTOR VDR 1,25-DIHYDROXYVITAMIN D3 RECEPTOR
AT motif-binding factor ATBF1
GA-binding protein beta 2 subunit GABP beta 2 subunit; GABPB2; GABPB
transcription termination factor 1 TTF1
bone morphogenic protein receptor type 1B BMPR1B; serine/threonine-protein kinase receptor 6 SKR6; a
NADPH-cytochrome P450 reductase CPR; POR
Vascular endothelial growth factor precursor VEGF, vascular permeability factor VPF
C-C chemokine receptor type 1 C-C CKR-1; CCR-1; macrophage inflammatory protein-1 alpha receptor MIP
sine oculis-related homeobox protein 4 homolog SIX4; skeletal muscle-specific ara-binding protein AR
activator of apoptosis harakiri HRK; neuronal death protein 5 DP5; BID3
ryocyte enhancer factor 2B MEF2B
gap junction alpha 1 protein GJA1; connexin 43 CXN43; CX43; gap junction 43-kDa heart protein
interleukin-1 beta precursor IL-1 beta; IL1B
interleukin 10
retinoic acid receptor alpha RAR-alpha; RARA
friend of GATA 1 FOG; ZFPM1
distal-less homeobox protein 2 DLX2; tes1
RET-box containing gene 3 Sox3
macrophage inflammatory protein 2 alpha MIP2-alpha
cytokine receptor common gamma subunit precursor gamma-c; interleukin-2 receptor gamma subunit IL-2R
43-kDa postsynaptic protein; acetylcholine receptor-associated 43-kDa protein; RAPSYN
homeobox protein A2 HOXA2; HOX-1.11
membrane metallo endopeptidase
androgen receptor
erythroid transcription factor NF-E2
triacetraproline TTP; tissue plasminogen activator-induced sequence 11 TPA-induced sequence 11 TIP1
epin in A2 precursor EFNA2; eph-related receptor tyrosine kinase ligand 6 EPLG2; LERK6; ELF1; CDK7 1
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FIG. 2. (A) Cluster analysis of gene upregulated by LED when compared to the untreated control in the tissue obtained from the sponge section of the wound of diabetic mouse model. Red color indicates upregulation of genes, while brighter color indicates higher fold changes. (B) Cluster analysis of downregulated genes in both time points after LED treatment. Green color indicated downregulation of genes, while brighter color indicates higher fold change compared to the dull green color.
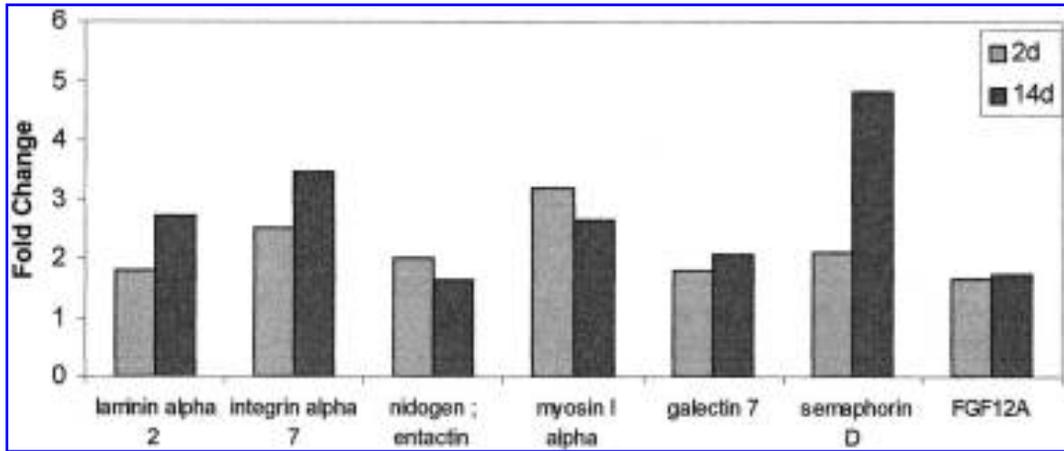
FIG. 3. Expression pattern of a few selected genes induced by LED at both time points in the sponge site of the wound.

Expression of basement membrane components occurs during sequential phases of wound healing and angiogenesis. Nidogen is one such protein along with gap junction proteins, actin that were upregulated by LED treatment. Laminin and nidogen transcripts are greatest during the early proliferative-migratory phase of angiogenesis but decrease significantly in later phases, when vessel maturation and tube formation predominate. There are reports that suggest that wound-induced epithelial cell migration is a finely tuned process that is dependent upon the regulated function and localization of specific laminins and their integrin receptors.[22]

Integrin alpha 7 beta 1 is a specific cellular receptor for the basement membrane protein laminin-1, as well as for the laminin isoforms -2 and -4. The alpha 7 subunit is expressed mainly in skeletal and cardiac muscle and has been suggested to be involved in differentiation and migration processes during myogenesis. Both integrins and laminins were among the many upregulated genes upon LED treatment when compared to the untreated controls. Principal stages of epidermal wound healing in human skin implies a linkage between BM assembly, integrin distribution and the compartment of proliferation competent cells, which in turn determines the onset of differentiation. Thus, apart from the balance of diffusible growth regulators, there is positional control of keratinocytes, largely accomplished by integrin–matrix interactions, which seems to be prerequisite to establishment and maintenance of tissue homeostasis.[23]

Homeobox genes are another family of genes, which were altered by LED treatment. Hox 7 and Hox 8 genes are known to play a role for the msh-like family of genes in mesodermal and muscle differentiation and patterning and may act as a key factor up-regulating a variety of proangiogenic stimuli.[24] The formation of new blood vessels from pre-existing blood vessels is thought to be critical for wound repair.

We have identified semaphorins/collapsins to be markedly increased upon exposure to LED that may in turn decrease pain. Mouse semaphorin H functions as a chemorepellent to guide or block sensory peripheral nerve ingrowth, most likely via neuropilin as a receptor.[25] With the increase of semaphoring D at the site of the wound, nerve growth would likely be directed to occur around, rather than through the wound area. Numerous studies have shown that pain slows the healing process probably due to CNS-directed recruitment of inflamma-
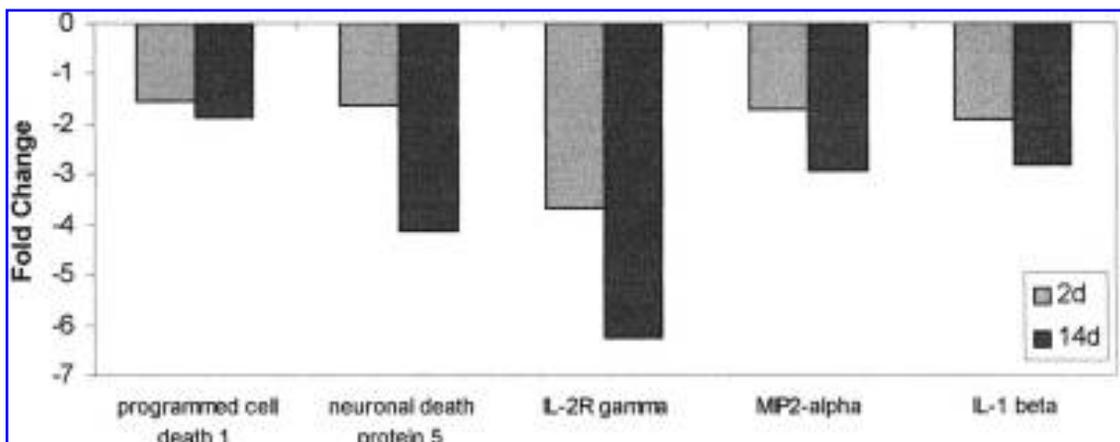

FIG. 4. Selected genes that were downregulated at both time periods in the sponge site of the wound upon LED treatment.

tory cells to the site of injury and their subsequent release of cytokines/eicosanoids and other mediators.

A cluster of calcium binding proteins was altered upon LED treatment. Calpactins are a family of related Ca^{2+}-regulated cytoskeletal proteins that were upregulated upon LED treatment. The calpactin I complex is composed of two heavy chain (39 K) and two light chain (11 K) subunits. The heavy chain is a member of a protein family that includes lipocortins, endonexin, and chromobindins, while the light chain is a member of the S100 family (seven distinct members are known). Many new members of the S-100 genes are known to be associated with cell differentiation, malignant transformation, and cell cycle. The messenger RNA levels of Calpactins have been reported to increase parallel to the S phase population of cells. Calpactins I and II are proteins that bind Ca^{2+}, phospholipids, actin and spectrin; they are also major substrates of oncogene and growth-factor-receptor tyrosine kinases.

Transforming growth factor–beta (TGF-beta), a potent regulator of wound healing and scar formation, is thought to have a key role in the response to injury.[26] TSP-1 promotes angiogenesis in the rat aorta model. TSP-1 has a predominant role in the activation of latent TGF-beta in malignant glioma cells.[27] TSP-1 is known to up-regulate the plasminogen activator system through a mechanism involving the activation of TGF-beta 1.[28] Both TGF beta-1 and TSP-1 were upregulated by 14 days of LED treatment in the current study suggesting they play an important role in the wound healing process.

A large number of proapoptotic genes along with cytokines and their receptors were downregulated by LED. Activator of apoptosis harakiri (HRK), programmed cell death 1 protein precursor (PDCD-1; PD-1) and RIP were among the many genes involved in apoptosis that were inhibited by LED. Receptor-interacting protein (RIP), a Ser/Thr kinase component of the tumor necrosis factor (TNF) receptor–1 signaling complex, mediates activation of the nuclear factor kappaB (NF-kappaB) pathway.[29] RIP2 has a C-terminal death domain, and RIP2, which has a C-terminal caspase activation and recruitment domain.[30] These cell death-associated genes were downregulated upon LED treatment in the mouse model, which suggests that there is increased proliferation induced by LED.

CONCLUSION

Using gene discovery techniques, one can begin to understand the biochemical mechanisms that are triggered by LED and may be playing a role in ultimately enhancing the healing process. LED effects the expression of genes involved in wound healing and possibly pain modulation thus enhancing the healing process. This work will directly lead to improvements in manipulating basic mechanisms to enhance rapid LED healing of acute combat trauma.

ACKNOWLEDGMENTS

REFERENCES

1. Conlan, M.J., Rapley, J.W., and Cobb, C.M. (1996). Biostimulation of wound healing by low-energy laser irradiation. J. Clin. Periodont. 23, 492–496.

2. Beauvoit, B., Kitai, T., and Chance B. (1994). Correlation between the light scattering and the mitochondrial content of normal tissues and transplantable rodent tumors. Biophys. J. 67, 2501–2510.

3. Beauvoit, B., Evans, S.M., Jenkins, T.W., et al. (1995). Contribution of the mitochondrial compartment to the optical properties of the rat liver: a theoretical and practical approach. Anal. Biochem. 226, 167–174.

4. Abergel, R.P., Lyons, R.F., Castel, J.C., et al. (1987). Biostimulation of wound healing by lasers: experimental approaches in animal models and in fibroblast cultures. J. Dermatol. Surg. Oncol. 13, 127–133.

5. Cooper, C.E., and Springett, R. (1997). Measurement of cytochrome oxidase and mitochondrial energetics by near-infrared spectroscopy. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 352, 669–676.

6. Mester, A.F., Nagylucskay, S., Mako, E., et al. (1998). Experimental imunological study with radiological application of low power laser. Laser Med. 509–512.

7. Mester, E., and Jaszsagi-Nagy, E. (1973). The effects of laser radiation on wound healing and collagen synthesis. Studia Biophys. 35, 227–230.

8. Lubart, R., Wollman, Y., Friedman, H., et al. (1992). Effects of visible and near-infrared lasers on cell cultures. J. Photochem. Photobiol. 12, 305–310.

9. Miller, M., and Truhe T. (1993). Lasers in dentistry: an overview. J. ADA 124, 32–35.

10. Yu, W., Naim, J.O., and Lanzafame, R.J. (1994). The effect of laser irradiation on the release of bFGF from 3T3 fibroblasts. Photochem. Photobiol. 59, 167–170.

11. Whelan, H.T., Houle, J.M., Donohoe, D.L., et al. (1999). Medical applications of space light-emitting diode technology—space station and beyond. Space Tech. App. Int. Forum 458, 3–15.

12. Whelan, H.T., Houle, J.M., Whelan, N.T., et al. (2000). The NASA light-emitting diode medical program-progress in space flight and terrestrial applications. Space Tech. App. Int. Forum 504, 37–43.

13. Whelan, H.T., Smits RL, Buchmann, E.V., et al. (2001). Effect of NASA light-emitting diode (LED) irradiation on wound healing. J. Clin. Laser Med. Surg. 19, 305–314.

14. Sommer, A.P., Pinheiro, A.L.B., Mester, A.R., et al. (2001). Biostimulatory windows in low intensity laser activation: lasers, scanners and NASA's light-emitting diode array system. J. Clin. Laser Med. Surg. 19, 29–34.

15. Bibikova, A., and Oron, U. (1995). Regeneration in denervated toad *(Bufo viridis)* gastrocnemius muscle and the promotion of the process by low energy laser irradiation. Anat. Rec. 241, 123–128.

16. Al-Watban, F.A. (1997). Laser acceleration of open skin wound closure in rats and its dosimetric dependence. Lasers Life Sci. 7, 237–247.

17. Karu, T. (1989). Photochemical effects upon the cornea, skin and other tissues (photobiology of low-power laser effects). Health Phys. 56, 691–704.

18. Chance, B., Nioka, S., Kent, J., et al. (1988). Time-resolved spectroscopy of hemoglobin and myoglobin in resting and ischemic muscle. Anal. Biochem. 174, 698–707.

19. Das R, C. Mendis, Z. Yan, et al. (1998). Alterations in gene expression show unique patterns in response to toxic agents. Presented at the 21st Army Science Conference.

20. Eisen, M.B., Spellman, P.T., Brown, P.O., et al. (1998). Cluster analysis and display of genome-wide expression patterns. Proc. Natl. Acad. Sci. U.S.A. 95, 14863–14868.

21. Yu, W., Naim, J.O., and Lanzafame, R.J. (1997). Effects of photostimulation on wound healing in diabetic mice. Lasers Surg. Med. 20, 56–63.

22. Lotz, M.M., Nusrat, A., Madara, J.L., et al. (1997). Intestinal epithelial restitution. Involvement of specific laminin isoforms and integrin laminin receptors in wound closure of a transformed model epithelium. Am. J. Pathol. 150, 747–760.

23. Breitkreutz, D., Stark, H.J., Mirancea, N., et al. (1997). Integrin and basement membrane normalization in mouse grafts of human keratinocytes—implications for epidermal homeostasis. Differentiation 61, 195–209.

24. Izpisua-Belmonte, J.C., and Duboule, D. (1992). Homeobox genes and pattern formation in the vertebrate limb. Dev. Biol. 152, 26–36.

25. Miyazaki, N., Furuyama, T., Amasaki, M., et al. (1999). Mouse semaphorin H inhibits neurite outgrowth from sensory neurons. Neurosci. Res. 33, 269–274.

26. Sinha, S., Heagerty, A.M., Shuttleworth, C.A., et al. (2002). Expression of latent TGF-beta binding proteins and association with TGF-beta 1 and fibrillin-1 following arterial injury. Cardiovasc. Res. 53, 971–983.

27. Sasaki, A., Naganuma, H., Satoh, E., et al. (2001). Participation of thrombospondin-1 in the activation of latent transforming growth factor–beta in malignant glioma cells. Neurol. Med. Chir. (Tokyo) 41, 253–258.

28. Yevdokimova, N., Wahab, N.A., and Mason, R.M. (2001). Thrombospondin-1 is the key activator of TGF-beta1 in human mesangial cells exposed to high glucose. J. Am. Soc. Nephrol. 12, 703–712.

29. Sun, X., Yin, J., Starovasnik, M.A., et al. (2002). Identification of a novel homotypic interaction motif required for the phosphorylation of receptor-interacting protein (RIP) by RIP3. J. Biol. Chem. 277, 9505–9511.

30. Holler, N., Zaru, R., Micheau, O., et al. (2000). Fas triggers an alternative, caspase-8–independent cell death pathway using the kinase RIP as effector molecule. Nat. Immunol. 1, 489–495.

31. Abergel, R.P., Lyons, R.F., Castel, J.C., et al. (1987). Biostimulation of wound healing by lasers: Experimental approaches in animal models and in fibroblast cultures. J. Dermatol. Surg. Oncol. 13, 127–133.

32. Al-Watban, F.A., and Zhang X.Y. (1991). Comparison of wound healing process using argon and krypton lasers. Biochem. Biophys. Acta 1091, 140–144.

33. Barasch, A., Peterson, D.E., Tanzer, J.M., et al. (1995). Helium-neon laser effects on conditioning-induced oral mucositis in bone marrow transplantation patients. Cancer 76, 2550–2556.

34. Cowen, D., Tardieu, C., Schubert, M., et al. (1997). Low energy helium-neon laser in the prevention of oral mucositis in patients undergoing bone marrow transplant: results of a double blind random trail. Int. J. Radiat. Oncol. Biol. Phys. 38, 697–703.

35. Eggert, H.R., and Blazek, V. (1993). Optical properties of normal human brain tissues in the spectral range of 400 to 2500 nm. Adv. Exp. Med. Biol. 333, 47–55.

36. Hartmann, K.M. (1983). Action spectroscopy. In: W. Hoppe, W. Lohmann, H. Marke, H. Ziegler (eds.) Biophysics, New York: Springer-Verlag, pp. 115–144.

37. Karu, T.I., Pyatibrat, L., and Kalendo, G. (1994). Irradiation with HeNe laser can influence the cytotoxic response of HeLa cells to ionizing radiation. Int. J. Radiat. Biol. 65, 691–704.

38. Lubart, R., Friedman, H., Sinyakov, M., et al. (1997). Changes in calcium transport in mammalian sperm mitochondria and plasma membranes caused by 780 nm irradiation. Lasers Surg. Med. 21, 493–499.

39. Mester, E., Nagylucskay, S., Triza, S., et al. (1978). Stimulation of wound healing by means of laser rays. Acta Chir. Acad. Sci. Hung. 19, 163–170.

40. Mester, E., Spivy, T., Szende, B., et al. (1971). Effect of laser rays on wound healing. Am. J. Surg. 122, 532–535.

41. Peterkofsky, B., and Diegelmann, R. (1971). Biochemistry 10, 988–994.

42. Salansky, N. (1998). Low energy photon therapy for wound healing. Intnl. Med. Instr., Canadian Defense Ministry, personal communication.

43. Schmidt, M.H., Bajic, D.M., Reichert, K.W. II, et al. (1996). Light-emitting diodes as a light source for intra-operative photodynamic therapy. Neurosurgery 38, 552–556.

44. Schmidt, M.H., Reichert, K.W. II, Ozker, K., et al. (1999). Preclinical evaluation of benzoporphyrin derivative combined with a light-emitting diode array for photodynamic therapy of brain tumors. Pediatr. Neurosurg. 30, 225–231.

45. Schubert, M.M., Williams, B.E., Lloid, M.E., et al. (1992). Clinical assessment scale for the rating of oral mucosal changes associated with bone marrow transplantation; development of an oral mucositis index. Cancer 69, 2469–2477.

46. Whelan, H.T., Schmidt, M.H., Segura, A.D., et al. (1993). The role of photodynamic therapy in posterior fossa brain tumors: a preclinical study in a canine glioma model. J. Neurosurg. 79, 562–568.

47. Yamada, K. (1991). Biological effects of low power laser irradiation on clonal osteoblastic cells (MC3T3-E1). J. Jpn. Orthop. Assoc. 65, 787–799.

Address reprint requests to:
Harry T. Whelan, M.D.
Department of Neurology
Medical College of Wisconsin
Milwaukee, WI 53226

E-mail: hwhelan@mcw.edu