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Ying-Ying Huang
Wellman Center for Photomedicine, Massachusetts General Hospital; Department of Dermatology, Harvard Medical School, Boston MA; and Aesthetic and Plastic Center of Guangxi Medical University, China

Sulbha K Sharma
Wellman Center for Photomedicine, Massachusetts General Hospital

James Carroll
THOR Photomedicine Ltd, UK

Michael R Hamblin
Wellman Center for Photomedicine, Massachusetts General Hospital; Department of Dermatology, Harvard Medical School; Harvard- MIT Division of Health Sciences and Technology

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BIPHASIC DOSE RESPONSE IN LOW LEVEL LIGHT THERAPY – AN UPDATE

Ying-Ying Huang □ Wellman Center for Photomedicine, Massachusetts General Hospital; Department of Dermatology, Harvard Medical School, Boston MA; and Aesthetic and Plastic Center of Guangxi Medical University, China

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James Carroll □ THOR Photomedicine Ltd, UK

Michael R Hamblin □ Wellman Center for Photomedicine, Massachusetts General Hospital; Department of Dermatology, Harvard Medical School; Harvard-MIT Division of Health Sciences and Technology

□ Low-level laser (light) therapy (LLLT) has been known since 1967 but still remains controversial due to incomplete understanding of the basic mechanisms and the selection of inappropriate dosimetric parameters that led to negative studies. The biphasic dose-response or Arndt-Schulz curve in LLLT has been shown both in vitro studies and in animal experiments. This review will provide an update to our previous (Huang *et al.* 2009) coverage of this topic. In vitro mediators of LLLT such as adenosine triphosphate (ATP) and mitochondrial membrane potential show biphasic patterns, while others such as mitochondrial reactive oxygen species show a triphasic dose-response with two distinct peaks. The Janus nature of reactive oxygen species (ROS) that may act as a beneficial signaling molecule at low concentrations and a harmful cytotoxic agent at high concentrations, may partly explain the observed responses in vivo. Transcranial LLLT for traumatic brain injury (TBI) in mice shows a distinct biphasic pattern with peaks in beneficial neurological effects observed when the number of treatments is varied, and when the energy density of an individual treatment is varied. Further understanding of the extent to which biphasic dose responses apply in LLLT will be necessary to optimize clinical treatments.

Keywords: low level laser therapy, photobiomodulation, biphasic dose response, reactive oxygen species, nitric oxide, traumatic brain injury

INTRODUCTION

Low level laser (light) therapy (LLLT) employs visible (generally red) or near-infrared light generated from a laser or light emitting diode (LED) system to treat diverse injuries or pathologies in humans or animals. The light is typically of narrow spectral width between 600nm - 1000nm. The fluence (energy density) used is generally between 1 and 20 J/cm^2 while the irradiance (power density) can vary widely depending on

Address correspondence to Prof. Michael R. Hamblin, BAR414, Massachusetts General Hospital, 40 Blossom Street, Boston, MA 02114; Ph: 617-726-6182; Fax:617-726-8566; email: Hamblin@helix.mgh.harvard.edu

the actual light source and spot size; values from 5 to 50 mW/cm^2 are common for stimulation and healing, while much higher irradiances (up to W/cm^2) can be used for nerve inhibition and pain relief. LLLT is typically used to promote tissue regeneration, reduce swelling and inflammation and relieve pain and is often applied to the injury for 30 seconds to a few minutes or so, a few times a week for several weeks. Unlike other medical laser procedures, LLLT is not an ablative or thermal mechanism, but rather a photochemical effect comparable to photosynthesis in plants whereby the light is absorbed and exerts a chemical change.

Within a decade of the introduction of LLLT in the 1970s it was realized that more does not necessarily mean better. The demonstration of the biphasic dose response curve in LLLT has been hampered by disagreement about exactly what constitutes a "dose". Many practitioners concentrate on fluence as the principle metric of dose, while others prefer irradiance or illumination time. The use of very small spot sizes by some practitioners has led to the assertion that they delivered hundreds of mW/cm^2 from a 50 mW laser. While this statement is mathematically correct it can give the impression that much higher doses of light were given than actually were delivered.

Two years ago we reviewed (Huang *et al.* 2009) the biphasic dose response in LLLT and found many reports in the literature concerning biphasic dose responses observed in cell cultures, some in animal experiments but no clinical reports. We now believe that the time is right to revisit this interesting topic for two reasons. Firstly because we have found more instances in our laboratory both in vitro with cultured cortical neurons, and in vivo with LLLT of traumatic brain injuries in mouse models. Secondly because advances have been made in mechanistic understanding of how LLLT works at a cellular level that may explain why a little light may be beneficial and at the same time a lot of light might be harmful.

MECHANISMS OF LOW LEVEL LIGHT THERAPY.

Basic photobiophysics and photochemistry

According to the First Law of Photochemistry, the photons of light must be absorbed by some molecular photoacceptors or chromophores for photochemistry to occur (Sutherland 2002).The mechanism of LLLT at the cellular level has been attributed to the absorption of monochromatic visible and near infrared (NIR) radiation by components of the cellular respiratory chain (Karu 1989). Phototherapy is characterized by its ability to induce photobiological processes in cells. The effective tissue penetration of light and the specific wavelength of light absorbed by photoacceptors are two of the major parameters to be considered in light therapy. In tissue there is an "optical window" that runs approximately from 650 nm to 1200 nm where the effective tissue penetration of light is

maximized. Therefore the use of LLLT in animals and patients almost exclusively involves red and near-infrared light (600-1100-nm) (Karu and Afanas'eva 1995). The action spectrum (a plot of biological effect against wavelength) shows which specific wavelengths of light are most effectively used for biological endpoints as well as for further investigations into cellular mechanisms of phototherapy (Karu and Kolyakov 2005). Fluence (J/cm^2) is often referred to as "dose", though many authors and practitioners of LLLT also refer to energy (Joules) as dose. Not only is this confusing to the novice student of LLLT but it also assumes that the product of power and time (and more importantly power density and time) is the goal rather than the right combination of individual values. This lack of reciprocity has been shown many times before and since our first paper on biphasic dose response and several more authors have reported finding these effects since. Examples of recently published "dose-rate" effects are also reviewed later in this article.

Mitochondrial Respiration and Cytochrome c oxidase

Mitochondria play an important role in energy generation and metabolism and are involved in current research about the mechanism of LLLT effects. The absorption of monochromatic visible and NIR radiation by components of the cellular respiratory chain has been considered as the primary mechanism of LLLT at the cellular level (Karu 1989). Cytochrome c oxidase (Cco) is proposed to be the primary photoacceptor for the red-NIR light range in mammalian cells. Absorption spectra obtained for biological responses to light were found to be very similar to the absorption spectra of Cco in different oxidation states (Karu and Kolyakov 2005).LLLT on isolated mitochondria increased proton electrochemical potential, ATP synthesis (Passarella *et al.* 1984), increased RNA and protein synthesis (Greco *et al.* 1989) and increases in oxygen consumption, mitochondrial membrane potential, and enhanced synthesis of NADH and ATP.

ROS release and Redox signaling pathway

Mitochondria are an important source of reactive oxygen species (ROS) within most mammalian cells. Mitochondrial ROS may act as a modulatable redox signal, reversibly affecting the activity of a range of functions in the mitochondria, cytosol and nucleus. ROS are very small molecules that include oxygen ions such as superoxide, free radicals such as hydroxyl radical, hydrogen peroxide, and organic peroxides. ROS are highly reactive with biological molecules such as proteins, nucleic acids and unsaturated lipids. ROS are also involved in the signaling pathways from mitochondria to nuclei. It is thought that cells have ROS or redox sensors whose function is to detect potentially harmful levels of ROS that

may cause cell damage, and then induce expression of anti-oxidant defenses such as superoxide dismutase and catalase.

LLLT was reported to produce a shift in overall cell redox potential in the direction of greater oxidation (Karu 1999) and increased ROS generation and cell redox activity have been demonstrated (Lubart *et al.* 2005). These cytosolic responses may in turn induce transcriptional changes. Several transcription factors are regulated by changes in cellular redox state, but the most important one is nuclear factor κB (NF-κB). Figure 1 graphically illustrates some of the intracellular signaling pathways that are proposed to occur after LLLT.

NO release and NO signaling

There have been reports of the production and/or release of NO from cells after in vitro LLLT. It is possible that the delivery of low fluences of red/NIR light produces a small amount of NO from mitochondria by dissociation from intracellular stores (Shiva and Gladwin 2009), such as nitrosothiols (Borutaite *et al.* 2000), NO bound to hemoglobin or myoglobin (Lohr *et al.* 2009; Zhang *et al.* 2009) or by dissociation of NO from Cco (Lane 2006) as depicted in Figure 2. A second mechanism for

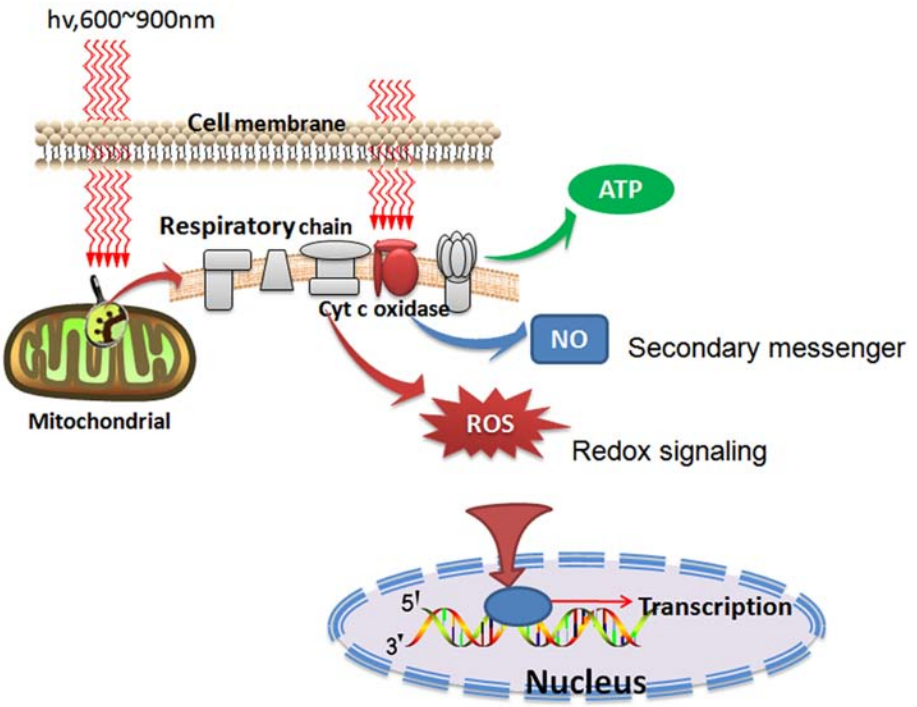

FIG. 1. Schematic depiction of the cellular signaling pathways triggered by LLLT. After photons are absorbed by chromophores in the mitochondria, respiration and ATP is increased but in addition signaling molecules such as reactive oxygen species (ROS) and nitric oxide (NO) are also produced.

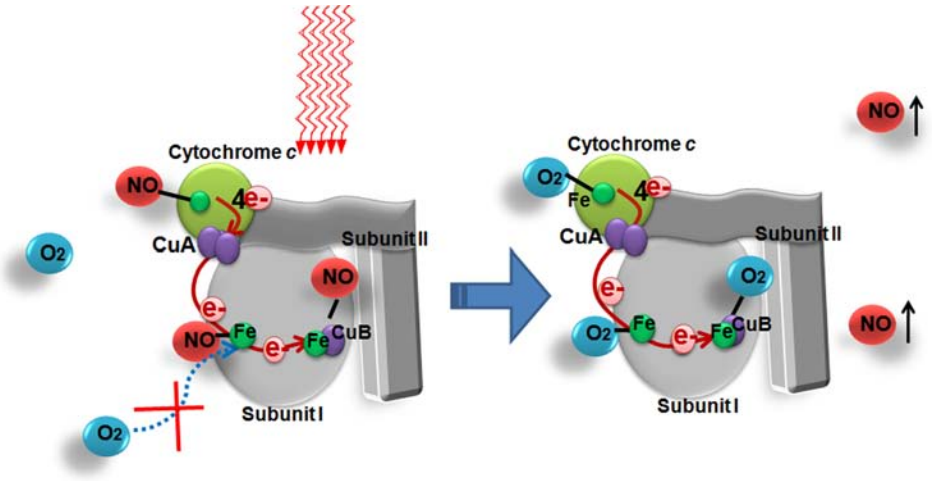

FIG. 2. One possible theory that can explain the simultaneous increase in respiration an production of nitric oxide is the photodissociation of bound NO that is inhibiting cytochrome c oxidase by displacing oxygen.

NO production is by light-mediated increase of the nitrite reductase activity of cytochrome c oxidase (Lane 2006). A third possibility is that light can cause increase of the activity of an isoform of nitric oxide synthase (Poyton and Ball 2011), possibly by increasing intracellular calcium levels. This low concentration of NO produced by illumination is proposed to be beneficial through cell-signaling pathways (Ball *et al.* 2011).

BIPHASIC DOSE RESPONSES IN LLLT

Many reports of biphasic dose responses in LLLT were reviewed in our previous contribution and for convenience we have assembled these reports into Tables. Table 1 lists reports on cultured cells in vitro, Table 2 lists those reports in animal models in vivo, while Table 3 contains the only report of biphasic dose response in clinical studies.

Figure 3 shows a 3D depiction of the Arndt Schulz model to illustrate a possible dose "sweet spot" at the target tissue. This graph suggests that insufficient power density or too short a time will have no effect on the pathology, that too much power density and / or time may have inhibitory effects and that there may be an optimal balance between power density and time that produces a maximal beneficial effect. There even may be a (low) power density for which infinite irradiation time would only have positive effects and no inhibitory effect. We believe that the absolute figures will be different at different wavelengths, tissue types, redox states, and may be affected further by different pulse parameters.

TABLE 1. Biphasic dose response studies of LLLT in vitro.

Year	Cells	Laser characteristics	Fluence	Irradiance	Reference
1978	Lymphocytes in vitro		"threshold phenomenon"		Mester *et al.* 1978
1990	Macrophage cell lines (U-937)	820nm Laser; 120mW/cm^2; 2.4J/ cm^2 to 9.6J/cm^2	Cell proliferation: Maximum at 7.2J/cm^2 least at 9.6J/cm^2		Bolton *et al.* 1990
1991	Macrophage cell lines (U-937)	820nm Laser; 2.4J/cm^2 or 7.2J/cm^2; 400mW/ cm^2 or 800mW/ cm^2		cell proliferation increased at 400mW/ cm^2; Cell viability reduced at 800mW/cm^2	Bolton *et al.* 1991
1994	Human oral mucosal fibroblast cells	812nm laser; 4.5mW/cm^2;	Cell proliferation peak at 0.45 J/cm^2; less at 1.422J/cm^2		Loevschall and Arenholt-Bindslev 1994
2001	Chinese hamster ovary and human fibroblast cells	He-Ne laser;1.25 mW/cm^2; 0.06 to 0.6J/cm^2	Cell proliferation peak at 0.18 J/cm2; less at 0.6J/cm^2.		al-Watban and Andres 2001
2003	human fibroblast cells	628nm LED; 11.46 mW/cm^2; 0, 0.44, 0.88, 2.00, 4.40, and 8.68 J /cm^2	Cell proliferation maximum at 0.88 J/cm^2; reduced at 8.68 J/cm^2		Zhang *et al.* 2003
2005	Human HEP-2 and murine L-929 cell lines	670 nm LED; 5 J/cm^2 per treatment; Total 50J/cm^2/day; 1 to 4 treatments/day	Cell proliferation bigger at 2 treatments/day		Brondon *et al.* 2005
2005	Hela cells	wavelength range of 580–860 nm	DNA synthesis rate maximum at 0.1 J/cm^2 with 0.8 mW/cm^2		Karu and Kolyakov 2005
2005	Wounded fibroblasts	632.8nm laser; 2mW/cm^2; 0.5, 2.5, 5.0 or 10.0 J/cm^2	Cell proliferation maximum at a single dose of 2.5J/cm^2; Cellular damage at 10J/cm^2		Hawkins and Abrahamse 2005
2006	Wounded fibroblasts	632.8nm laser; 5.0 J/ cm^2 or 16J/ cm^2	Cell proliferation and cell viability increased at 5 J/cm^2; decreased at 10 and 16 J/cm^2		Hawkins and Abrahamse 2006a
2006	Wounded fibroblasts	632.8nm laser; 5.0 J/cm^2 or 16J/cm^2	Cell migration and proliferation increased at a single dose of 5.0 J/cm^2 and two or three doses of 2.5 J/cm^2; inhibited at 16 J/cm^2		Hawkins and Abrahamse 2006b
2007	Human Neural Progenitor Cells (NHNPCs)	810nm; 0.2J/ cm^2; 50mW/cm^2 and 100mW/ cm^2		Neurite outgrowth greater at 50mW/cm^2; less at 100mW/cm^2	Anders *et al.* 2007
2009	Rheumatoid arthritis synoviocytes	810nm laser_1, 3, 5, 10, 20 and 50 J/cm^2	Cell proliferation increased at 5 J/cm^2 (16.7 mW/cm^2); Lower at 50 J/cm^2		Yamaura *et al.* 2009
2009	Mouse embryonic fibroblasts	810nm laser; 0.003,0.03,0.3,3 or 30J/cm^2	NF-κB activation maximum at 0.3 J/cm^2; decreased at 3 J/cm^2 and 30 J/cm^2		Chen *et al.* 2009

TABLE 2. Biphasic dose response studies of LLLT in vivo (animal models).

Year	Tissue	Laser characteristics	Fluence	Irradiance	Reference
1979	wound closure	He-Ne laser4 J/cm^2		Wound healing best at 45 mW/cm^2; least at 12.4 mW/cm^2	Ginsbach 1979
2001	Induced heart attacks in rats	810 nm laser; 2.5 to 20mW/cm^2 ;		Reductions of infarct size maximum at 5mW/cm^2 Lower effects both at 2.5mW/cm^2 and 20mW/cm^2	Oron *et al.* 2001
2005	Mouse pleurisy induced by Carrageenan	650nm laser; 2.5 mW in 0.08 cm^2; 3 J/cm^2, 7.5 J/cm^2, and 15 J/cm^2	Inflammatory cell migration reduction most at 7.5 J/cm^2; Less at 3 and 15 J/cm^2		Lopes-Martins *et al.* 2005
2007	Healing of pressure ulcers in mice	670nm LED; 5 J/cm^2 at 0.7, 2, 8 or 40mW/cm^2		Healing significant improved only at 8mW/cm^2;Less at 0.7, 2, and 40 mW/cm^2	Lanzafame *et al.* 2007
2007	Full thickness dorsal excisional wound in BALB/c mice	a single exposure from 635, 670, 720 or 820nm filtered lamp; 1, 2, 10 and 50 J/cm^2; 100 mW/cm^2 10, 20, 100 and 500 seconds	Healing effect best at 2 J/cm^2 for 635nm light; worse at 50 J/cm^2 for most wavelengths compared to no treatment	820nm was the best wavelength	Demidova-Rice *et al.* 2007
2007	Inflammatory arthritis induced by zymosan in rats	810-nm laser; 3 and 30 J/cm^2; 5 mW/cm^2 and 50 mW/cm^2	30 J/cm^2 was better than 3 J/cm^2 at 50mW/cm^2	3 J/cm^2 has effective at 5mW/cm^2 but not 50mW/cm^2	Castano *et al.* 2007

TABLE 3. Biphasic dose response studies of LLLT in clinical studies.

Year	Patients	Laser characteristics	Fluence	Irradiance	Reference
1997	Patients with post herpetic neuralgia of the facial type	830nm lasers; 60mW laser and 150mW laser; irradiance point at 4mm in diameter		Pain reduction greater at 150mW laser; less at 60mW laser when exposure to the same time.	Hashimoto *et al.* 1997

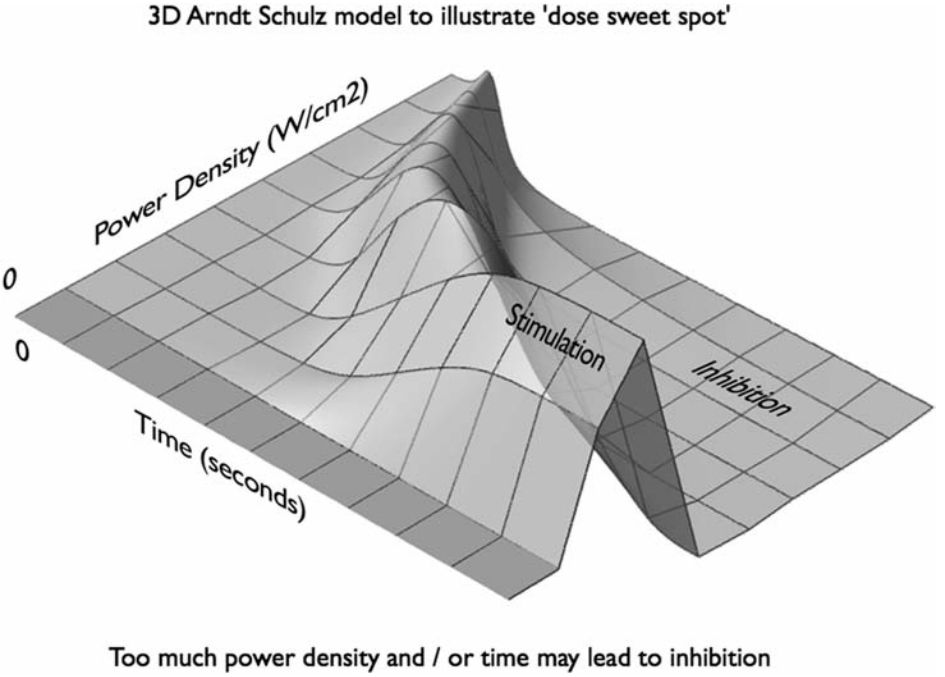

FIG. 3. Three-dimensional model of the Arndt-Schulz curve illustrating how either irradiance or illumination time (fluence) can have biphasic dose response effects in LLLT.

CURRENT BIPHASIC DOSE RESPONSE STUDIES IN LLLT.

In this section we cover the new reports of biphasic dose responses in LLLT that have been published in the last two years since our previous review.

In an oral mucositis hamster model Lopes and coworkers (Lopes *et al.* 2009) delivered 660-nm laser at two different irradiances (55 mW/cm^2 for 16 seconds per point or 155 mW/cm^2 for 6 seconds per point). Both regimens delivered 0.9 J/cm^2 per point. On day 7, 11 and 15 the authors reported reduced severity of clinical mucositis and lower levels of COX-2 staining in the 55 mW/cm^2 group and that the 155 mW/cm^2 had no significant differences when compared with controls. This data is summarized in Figure 4.

Gal et al (Gal *et al.* 2009) compared the effects of delivering 5 J/cm^2 of 670-nm laser at different power densities on wound tensile strength in a rat model. They found (Figure 5) that 670 nm laser achieved a significant effect using 4mW/cm^2 applied for 1,250 seconds (20 mins 50 seconds) but that this effect was lost if the same 5J/cm^2 fluence was delivered at 15 mW/cm^2 for 333 seconds (5 mins 33 seconds).

(Skopin and Molitor 2009) studied the effects of different influences of 980 nm laser on a human fibroblast in vitro model of wound healing.

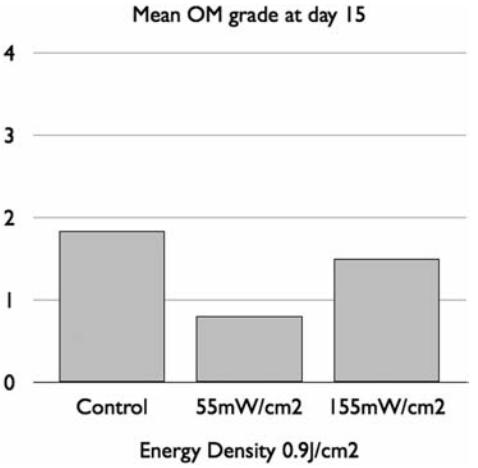

FIG. 4. Mean grading of oral mucositis (OM) in a hamster cheek pouch model treated with 0.9 J/cm^2 of 660-nm laser at two different irradiances (55 mW/cm^2 for 16 seconds per point or 155 mW/cm^2 for 6 seconds per point). Graph redrawn from data contained in (Lopes, Plapler et al. 2009).

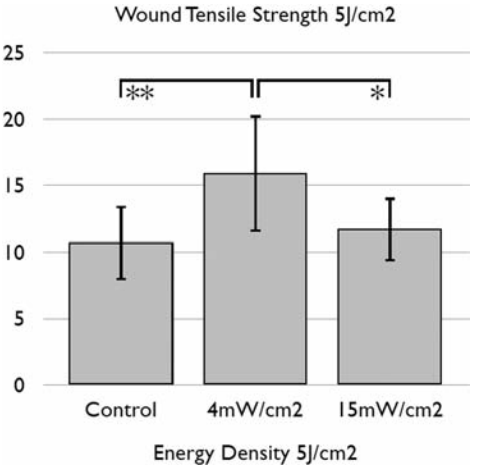

FIG. 5. Mean wound tensile strength obtained after delivering 5 J/cm^2 of 670-nm laser at different power densities (4mW/cm^2 applied for 1,250 seconds or 15 mW/cm^2 for 333 seconds). Graph redrawn from data contained in (Gal, Mokry et al. 2009).

A small pipette was used to induce a wound in fibroblast cell cultures, which were exposed to a range of laser doses (1.5-66 J/cm^2). Exposure to low- and medium-dose laser light accelerated cell growth, whereas high-intensity light negated the beneficial effects of laser exposure as shown in Figure 6.

(Prabhu *et al.* 2010) performed a dose response study by applying a 7 mW HeNe (632.8-nm) laser with a power density of 4 mW/cm^2 to 15×15 mm excisional wounds on Swiss albino mice for a range of irradiation times from 249 seconds (4.15 mins) up to 2,290 seconds (41.46 mins). As

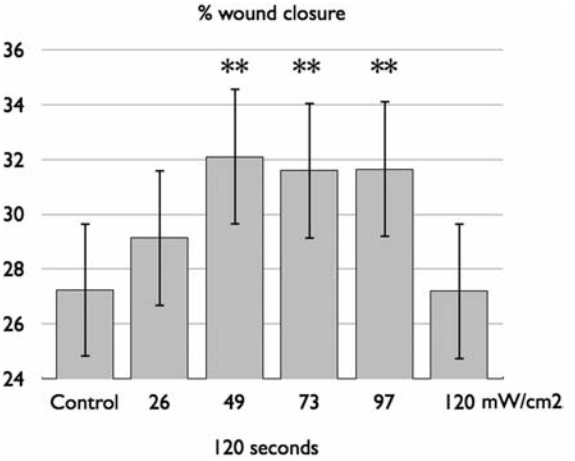

FIG. 6. Mean percentage of healing induced in a scratch wounded culture of human fibroblasts using different fluences (constant time, increasing irradiance) of 980-nm laser. Graph redrawn from data contained in (Gal, Mokry et al. 2009).

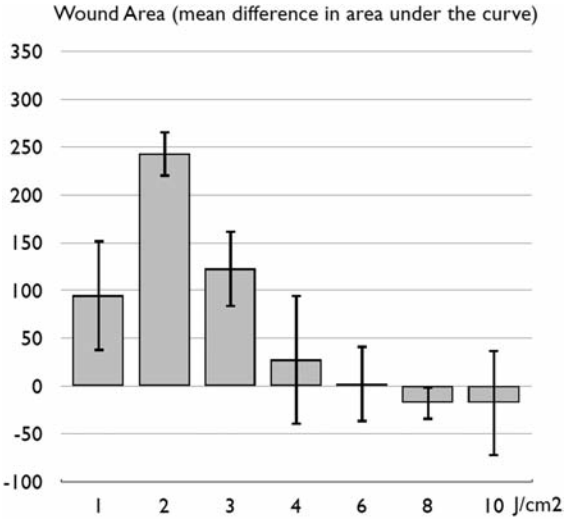

FIG. 7. Mean area under the curve of wound area over time in a mouse excisional wound healing model treated with a 7 mW (power density of 4 mW/cm^2) HeNe (632.8-nm) laser for times ranging from 249 to 2,290 seconds. Graph redrawn from data contained in (Prabhu, Rao et al. 2010).

Figure 7 shows, there was a clear biphasic response (including a possible inhibitory effect) with changes in irradiation time and therefore fluence.

BIPHASIC LLLT DOSE RESPONSE STUDIES IN CULTURED NEURONS AND TRAUMATIC BRAIN INJURY MODELS IN MICE.

LLLT studies on cultured cortical neurons

In order to elucidate the mechanism responsible for the beneficial effect reported by LLLT for brain related disorders, we carried out stud-

ies to look into effects of 810 nm laser on different cellular signaling molecules in primary cortical neurons. The primary cortical neurons were isolated from brains taken from embryonic mice. We irradiated the neurons with different fluences of 0.03, 0.3, 3, 10 or 30 J/cm^2 delivered at a constant irradiance of 25 mW/cm^2, and subsequently the intracellular levels of ROS, mitochondrial membrane potential (MMP) and ATP was measured. The changes in mitochondrial function were studied in terms of ATP and MMP. Low-level light was found to induce a significant increase in ATP and MMP at lower fluences and a decrease at higher fluence. ROS was induced significantly by light at all light doses but there was a distinctive pattern of a double peak with the first peak coinciding with the other peaks of ATP and MMP at 3 J/cm^2 (Figure 8). However in contrast to ATP and MMP there was a second larger rise in ROS at 30 J/cm^2 that coincided with the reduction in MMP below baseline. The results of the this study suggested that LLLT at lower fluences is capable of inducing mediators of cell signaling process which in turn may be responsible for the biomodulatory effects of the low level laser. Conversely at higher fluences beneficial mediators are reduced but potentially harmful mediators are increased. Thus this study offered an explanation for the biphasic dose response induced by LLLT.

LLLT in a mouse model of traumatic brain injury.

We have been studying the effect of transcranial laser (810-nm) on mouse models of traumatic brain injury. The model involves a controlled cortical impact using a pneumatic piston device through a craniotomy followed by closure of the head. This injury can be adjusted in severity to

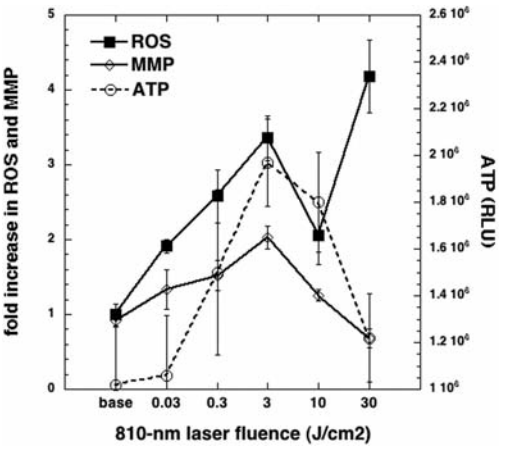

FIG. 8. Mean expression levels of reactive oxygen species (ROS, measured by MitoSox red fluorescence), mitochondrial membrane potential (MMP, measured by red/green fluorescence ration of JC1 dye) and ATP (measured by firefly luciferase assay) in primary mouse cortical neurons treated with various fluences of 810-laser delivered at 25 mW/cm^2 over times varying from 1.2 to 1200 seconds.

produce a neurological severity score (NSS based on a panel of standardized behavioral tests) of 7-8 on a scale of 0 (normal mice) to 10 (severe brain injury that causes death). The basic finding was that delivering a single dose of 36 J/cm^2 810-nm laser delivered at 50 mW/cm^2 (12 minutes illumination time) in a spot of 1-cm diameter centered on the top of the mouse head at a time point of 4 hours post-TBI was highly effective in ameliorating the neurological symptoms suffered by the mice (Figure 9A). When we delivered 10 times as much 810-nm laser (360 J/cm^2 at 500 mW/cm^2) also taking 12 minutes the beneficial effect totally disappeared, and at early time points (1-6 days) the high fluence appeared to be worse than no treatment (Figure 9B).

When we repeated the effective laser treatments 14 times (36 J/cm^2 delivered at 50-mW/cm^2 once a day for 14 days starting 4 hours post-TB) we found a very interesting result (Figure 9C). For the first 4 days the improvement in NSS in the repeated laser group was marginally better than the single treatment. However on day 5 the gradual improvement ceased and as the laser was repeated the NSS got closer to that of untreated TBI mice until at day 14 it actually crossed over. Although the differences were not statistically significant it appeared that from day 16 until day 28 the mice that received 14 laser treatments did worse than those that received no treatment at all.

POSSIBLE EXPLANATIONS FOR BIPHASIC DOSE RESPONSE IN LLLT

The triphasic dose response we have observed for ROS production in cultured cortical neurons (see Fig 7) suggests an explanation for the biphasic dose response. The hypothesis is that there are two kinds of ROS. Good ROS are produced at fairly low fluences of light. The reason for the production of good ROS is likely to be connected with stimulation of mitochondrial electron transport as shown by increases in MMP and increases in ATP production. These good ROS can initiate beneficial cell signaling pathwas leading to activation of redox sensitive transcription factors such as NF-κB (Chandel *et al.* 2000; Groeger *et al.* 2009). NF-κB activation induces expression of a large number of gene products related to cell proliferation and survival (Karin and Lin 2002; Brea-Calvo *et al.* 2009). As the fluence of light is increased the beneficial ROS production in the mitochondria decreases in tandem with reductions in MMP and a drop-off in ATP production. Then when even more light is delivered there is a second peak in ROS production, which we will call bad ROS. Bad ROS can damage the mitochondria leading to a drop in MMP below baseline levels and presumably can lead to initiation of apoptosis by the mitochondrial pathway including cytochrome c release. It remains to be seen whether the good and bad ROS are identical species and just differ in amount, or whether they are chemically different species. For instance it may be hypothesized that the good ROS consists mainly of superoxide

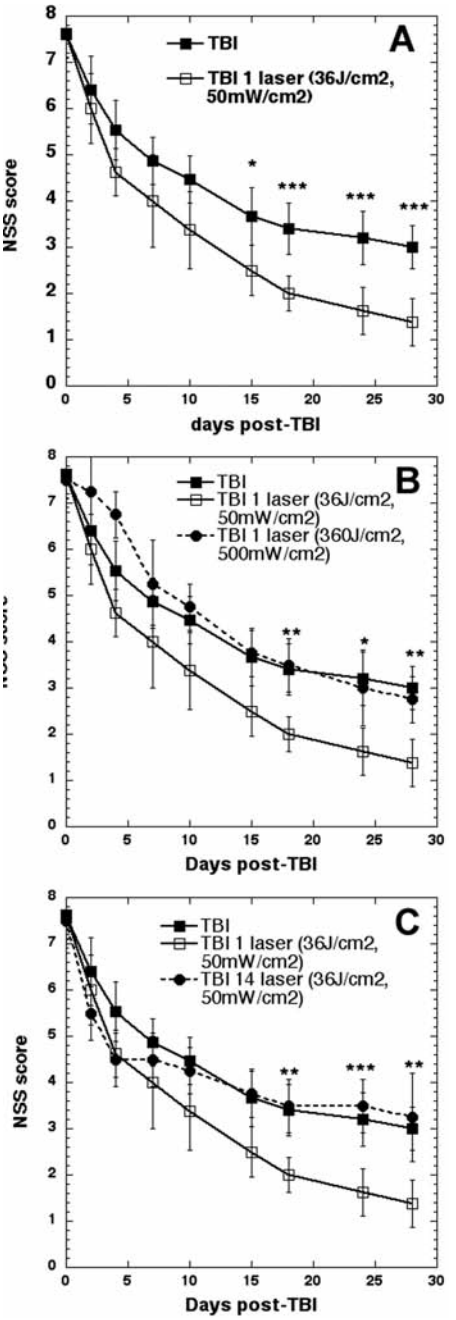

FIG. 9. Transcranial laser therapy (36 J/cm^2 of 810-nm laser delivered at 50 mW/cm^2 (12 minutes illumination time) in a spot of 1-cm diameter centered on the top of the mouse head) was used to treat mice with controlled cortical impact TBI four hours after injury. (A) Significant improvement in neurological severity score continuing for 4 weeks after a single treatment. (B) Delivering ten times more light by increasing irradiance tenfold (500 mW/cm^2) loses all therapeutic benefit, and produces worse performance soon after laser. (C) Repeating beneficial laser treatment daily for 14 days loses benefit in performance after 5 days.

while the bad ROS consists of more damaging ROS such as hydroxyl radicals and peroxynitrite. In Figure 7 we used just one type of fluorescent ROS indicator (mitoSOX red), which is commonly supposed to be specific for superoxide but will likely also be activated by hydroxyl radicals and peroxynitrite.

There have been several studies showing that relatively high doses of light can induce apoptosis in various cell types via ROS-mediated signaling pathways (Huang *et al.* 2011). Meanwhile, there is an important proapoptotic signaling pathway has been identified which involves Akt/GSK3beta inactivation after high-fluence low-power laser irradiation (HF-LPLI) (Huang, Wu et al. 2011). This research extended the knowledge of the biological mechanisms of cytotoxicity induced by HF-LPLI. In one of the studies it was shown that HF-LPLI does not activate caspase-8, indicating that the induced apoptosis was initiated directly from mitochondrial ROS generation and a decrease in MMP, independent of caspase-8 activation (Wu *et al.* 2007). Another study revealed HF-LPLI induced cell apoptosis via the CsA-sensitive MPT, which was ROS-dependent. They also showed a secondary signaling pathway through Bax activation. It was concluded that link between MPT and triggering ROS could be a fundamental phenomenon in HF-LPLI-induced cell apoptosis (Wu *et al.* 2009).

Further work is necessary to fully elucidate the molecular and cellular mechanisms responsible for the biphasic dose response in LLLT. Besides the role of ROS, which we have discussed above, the role of another Janus-type mediator, nitric oxide (NO) may play a role. If high fluences of light could produce high concentrations of NO, this might result in cytotoxicity via formation of peroxynitrite or other reactive nitrogen species (Hirst and Robson 2010).

SUMMARY AND CONCLUSION

The number of instances of biphasic dose response reported in the LLLT literature is increasing as time progresses. This increase may be due to an increasing realization that the phenomenon is real, and thus prompting investigators to look for it. At present there has been no convincing report of biphasic dose responses occurring in patients, but several systematic reviews and meta analyses of randomized controlled trials in LLLT have found (Bjordal *et al.* 2003; Tumilty *et al.* 2009) that some ineffective trials may be explained by over-dosing, in that the guidelines set by World Association for Laser Therapy (www.walt.nu) were exceeded. As more clinical trials of LLLT are reported there is an increasing likelihood that this unfortunate state of affairs will continue unless the dosimetry is designed to take into account the biphasic dose response phenomenon. Moreover it is unknown to what extent the parameters needed for the onset of the biphasic dose response will vary in a highly heteroge-

neous patient population, as compared with a highly uniform population of experimental animals (inbred lab animals are genetically identical).

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