

Low energy laser light (632.8 nm) suppresses amyloid- β peptide-induced oxidative and inflammatory responses in astrocytes

Xiaoguang Yang,^a Sholpan Askarova,^a Wenwen Sheng,^{b,c} JK Chen,^d Albert Y. Sun,^e Grace Y. Sun,^{b,c} Gang Yao,^a and James C-M. Lee^{a,*}

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Abstract

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INTRODUCTION

Oxidative stress and inflammation have been implicated in many neurodegenerative diseases including AD ([Behl et al., 1994](#), [Simonian and Coyle, 1996](#), [Behl and Holsboer, 1998](#), [Heneka and O'Banion, 2007](#)). Oxidative stress induced by overproduction of reactive oxygen species (ROS) causes damage of basic components in cells, such as lipids ([Cini and Moretti, 1995](#)), DNA ([Mecocci et al., 1994](#)) and proteins ([Smith et al., 1991](#)). In most cell systems, NADPH oxidase ([Abramov et al., 2004](#)) and mitochondrial abnormalities ([Hirai et al., 2001](#)) are two important sources of ROS. NADPH oxidase is comprised of six subunits ([Groemping and Rittinger, 2005](#), [Mizrahi et al., 2006](#)) and its activation is mediated by translocation of the cytosolic subunits (p47^{phox}, p67^{phox}, p40^{phox} and the GTPase Rac) to the plasma membrane subunits (gp91^{phox} and p22^{phox}) ([Groemping and Rittinger, 2005](#), [Mizrahi et al., 2006](#)). Activation of NADPH oxidase in astrocytes and microglia results in increased production of superoxide anions, which are toxic to neighboring neurons in AD brains ([Shimohama et al., 2000](#), [Qin et al., 2002](#), [Abramov et al., 2004](#)). Oxidative stress also triggers critical downstream pathways including activation of cPLA₂, an enzyme responsible for membrane integrity ([You et al., 2005](#), [Huber et al., 2006](#), [Sun et al., 2007](#), [Shelat et al., 2008](#)). Consistent with this line of evidence, we have reported that A β activates NADPH oxidase to induce ROS and activation of cPLA₂ in primary rat astrocytes ([Zhu et al., 2006](#)). Activated cPLA₂, in turn, targets mitochondria, resulting in mitochondrial dysfunction and further overproduction of ROS ([Zhu et al., 2006](#)). Based on these previous studies, it is reasonable to suggest two major mechanisms for A β to induce ROS production, initially from NADPH oxidase and subsequently from mitochondria through cPLA₂ activation.

There is compelling evidence that inflammation plays a vital role in pathogenesis of AD ([Heneka and O'Banion, 2007](#)). A β triggers expressions of inflammatory factors including IL-1 β and iNOS in glial cells ([Akama and Van Eldik, 2000](#), [White et al., 2005](#)). IL-1 β is a critical inflammatory cytokine in AD ([Griffin and Mrak, 2002](#)) since it can stimulate production of iNOS and other inflammatory cytokines ([Lee et al., 1993](#), [Blom et al., 1997](#)).

Low-level laser has been reported to attenuate oxidative stress and inflammation ([Karageuzyan et al., 1998](#), [Sakurai et al., 2000](#), [Freitas et al., 2001](#), [Fillipin et al., 2005](#), [Abdel et al., 2007](#), [Lim et al., 2007](#), [Aimbire et al., 2008](#), [Boschi et al., 2008](#), [Hammer et al., 2008](#), [Giuliani et al., 2009](#)). Although the mechanism is yet to be fully understood, this type of light therapy has been used to help tissue repair and wound healing in *in vivo* models ([Whelan et al., 2001](#), [Whelan et al., 2003](#), [Albertini et al., 2007](#), [Correa et al., 2007](#), [Viegas et al., 2007](#), [Aimbire et al., 2008](#), [Reis et al., 2008](#)) and rescue neurons from neurotoxic injuries ([Wong-Riley et al., 2005](#), [Liang et al.,](#)

2006), implying a variety of promising clinical applications. In this study, we hypothesize that laser has the capability of suppressing A β -induced oxidative stress and inflammation in astrocytes, the most abundant cell type in the brain. We tested the effects of low-level laser light at 632.8 nm on A β -induced ROS production through the activation of NADPH oxidase, and its downstream pathways involving phosphorylation of cPLA₂ and expression of inflammatory factors including IL-1 β and iNOS. Information derived from this study should prove to provide groundwork for further investigations on the potential application of laser therapy as a treatment for AD.

EXPERIMENTAL PROCEDURES

Chemicals and Reagents

Dulbecco's modified Eagle's medium (DMEM) with high glucose, Ham's F-12 medium, fetal bovine serum (FBS), dihydroethidium (DHE) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were from Invitrogen (Carlsbad, CA). Bovine serum albumin (BSA), dimethyl sulfoxide (DMSO), hexafluoro-2-propanol (HFIP) and poly-D-lysine were from Sigma-Aldrich (St. Louis, MO). A β ₁₋₄₂ was from American Peptide (Sunnyvale, CA). gp91 ds-tat Peptide 2, a peptide inhibitor of NADPH oxidase, was from anaSpec (Fremont, CA). Goat polyclonal anti-gp91^{phox} and rabbit polyclonal anti-p47^{phox} were from Santa Cruz Biotechnology (Santa Cruz, CA). Fluorescein-donkey anti-goat antibody, and Texas Red-sheep anti-rabbit antibody were from Abcam (Cambridge, MA).

Cell culture

Primary cortical astrocytes were obtained using a standard stratification/cell-shaking procedure from newborn rat brains. Following the procedure from our previous studies yielded confluent mixed glial cultures within 7–9 days, after which the flasks were shaken at 180 rev./min at room temperature (25°C) for 3h to remove microglial cells (Zhu et al., 2005, Zhu et al., 2006). The purity of these primary rat astrocyte cultures was >95% verified by anti-glial fibrillary acidic protein labeling (data not shown). Astrocytes were cultured onto 35mm dishes or coverslips coated with poly-D-lysine (0.4mg/ml) and fed every 48 h with fresh DMEM culture medium supplemented with 10% FBS. Cells were maintained at 37°C in a 5% CO₂ humidified incubator.

Preparation of A β ₁₋₄₂

A β ₁₋₄₂ (1mg) in the powder form was dissolved in 200 μ l of HFIP, and the solution was aliquoted into Eppendorf tubes, and after removing HFIP using a speed vacuum apparatus, samples were stored at –20°C until use. The A β film left in the tube was resuspended in 2 μ l DMSO and further diluted in 98 μ l Ham's F-12 medium to make a 100 μ M A β ₁₋₄₂ solution. The solution was then sonicated for 1 min and further diluted in DMEM to the final concentration of 5 μ M for treatments.

Laser irradiation protocol and A β treatment

The source of light for irradiation was a helium-neon laser (λ =632.8 nm) with an output power of 15mW. The light source was placed outside of incubator and an optical fiber was used to guide the laser from above onto a 35 mm dish covered with a plastic lid inside the incubator (Fig. 1A). Coverslips coated with poly-D-lysine were in 35 mm dishes if immunostaining was needed for

confocal microscopy. The spot size was adjusted to cover the whole dish with area of 10 cm². The irradiation power density was 1.5mW/cm². In this study, the irradiation time was 3 h. The total energy was 162 J and energy density was 16.2 J/cm². Both cell morphology and trypan blue exclusion assay showed that laser irradiation did not cause any ill-effect to cell viability (data not shown). In addition, no detectable change in culture medium temperature was observed during laser irradiation.

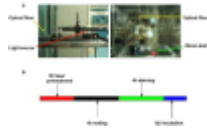


Fig. 1

Laser irradiation set-up and protocol

Fig. 1B describes the laser irradiation protocol. Briefly, for the group with laser pretreatment and A β treatment, astrocytes in 35mm dishes and DMEM with phenol red and 10% FBS were pretreated with laser for 3 h. After allowing cells to rest for another 4 h, cells were serum starved for 4 h by replacing DMEM containing 10% FBS with serum-free DMEM. Cells were then incubated with A β (5 μ M) for 2 h for measuring phosphorylation of cPLA₂, superoxide anion production, and colocalization between p47^{phox} and gp91^{phox}, and for 16 h for measuring IL-1 β and iNOS. For control group, astrocytes were starved for 4 h and incubated in serum-free DMEM. For the group with A β treatment alone, astrocytes were starved for 4 h and then incubated with A β in a CO₂-incubator without laser installed. For the group with treatment with NADPH oxidase inhibitor (gp91 ds-tat Peptide 2), the inhibitor (1 μ M) was added into culture medium 1 h before A β treatment.

Reactive oxygen species measurement

To quantify superoxide anion production induced by A β in astrocytes, cells were starved for 4 h, followed by incubation of cells with both A β (5 μ M) and DHE (20 μ M) for 2 h. DHE is a cell permeable fluorescent probe. Upon oxidation by superoxide anion, it binds to DNA in nuclei and becomes highly fluorescent ([Chapman et al., 2005](#)). Fluorescent intensity measurement of DHE was performed at room temperature using a Nikon TE-2000 U fluorescence microscope with a 20X objective lens. Images were acquired using a CCD camera controlled by a computer running MetaVue imaging software (Universal Imaging, PA). The fluorescent intensity of DHE per cell was measured. Background subtraction was done for all images prior to data analysis.

Confocal immunofluorescence microscopy

After treatments, cells were fixed with 4% paraformaldehyde at 37°C for 30 min. PBS containing 5% BSA was then applied to cells for 1 h to block nonspecific bindings. To label gp91^{phox} at the cell surface, goat polyclonal anti-gp91^{phox} (1:200 dilution) was added and incubated at 4°C without permeabilization. To label p47^{phox}, cells were then permeabilized with 0.1% Triton X-100 in PBS for 5 min. Rabbit polyclonal anti-p47^{phox} (1:200 dilution) in PBS with 1% BSA was then added and incubated at 4°C overnight. This was followed by fluorescent labeling with secondary antibodies (1:500 dilution) at room temperature for 1 h. The secondary antibodies for gp91^{phox} and p47^{phox} were fluorescein-donkey anti-goat antibody, and Texas Red-sheep anti-rabbit antibody, respectively. Secondary antibodies did not show immunostaining in the absence of the primary antibody (data not shown). Confocal immunofluorescence microscopy was performed with an Olympus FV1000 confocal inverted microscope (Tokyo, Japan). Confocal images were acquired with a 60X, numerical aperture 1.2 oil immersion

objective lens for colocalization studies between NADPH oxidase subunits, gp91^{phox} and p47^{phox}. Background subtraction was done for all images before analysis. Colocalization images were obtained by suppressing all colors, except yellow, in superimposed images using Adobe Photoshop (Adobe Systems, San Jose, CA). The colocalization of p47^{phox} with gp91^{phox} was quantified by normalizing the intensity of yellow by the intensity of gp91^{phox}.

Western blot analysis

After treatments, the total protein concentration of cell lysate was determined by BCA (bicinchoninic acid) protein assay kit (Pierce Biotechnology, Rockford, IL) according to manufacture's instruction. Equivalent amounts of protein from each sample (e.g., 40 µg) were diluted with Laemmli buffer, boiled for 5 min, subjected to electrophoresis in 7.5% SDS-polyacrylamide gels, and transferred to nitrocellulose membranes. Membranes were blocked for 1 h with 5% (w/v) nonfat dry milk in Tris-buffered saline containing 0.1% (v/v) Tween 20 (TBST) and were incubated overnight at 4°C in 3% (w/v) BSA with 0.02% (w/v) sodium azide in TBST with p-cPLA₂ or cPLA₂ antibodies (1:1000 dilution; Cell Signaling Technology, Beverly, MA), anti-IL-1β antibody (1:1000 dilution; R&D Systems, Minneapolis, MN), anti-iNOS antibody (1:1000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) and β-actin antibody (Sigma, St. Louis, MO). Membranes were washed three times during a 15-min period with TBST and incubated with horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit IgG antibody (1: 5000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) in 5% (w/v) nonfat dry milk in TBST at room temperature for 1 h. After washing with TBST for three times, the membrane was subjected to SuperSignal West Pico Chemiluminescent detection reagents from Pierce (Rockford, IL) to visualize bands. The protein bands detected on x-ray film were quantified using a computer-driven scanner and Quantity One software (Bio-Rad, Hercules, CA).

Statistical analysis

Data were presented as mean ± SD from at least three independent experiments. Analysis was carried out with one-way ANOVA, followed by *Bonferroni's* post hoc tests. Values of $p < 0.05$ are considered to be statistically significant.

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RESULTS

Laser light suppressed Aβ-induced superoxide production in primary astrocytes

In order to investigate the effects of laser light on Aβ-induced superoxide production, primary rat cortical astrocytes were fluorescently labeled with DHE. We found that stimulation of astrocytes by Aβ for 2 h led to an increase in superoxide anions, as indicated by a significant increase in DHE fluorescent intensity, whereas pretreatment of cells with laser abrogated an Aβ-mediated increase in superoxide anions ([Fig. 2](#)). Astrocytes exposed to laser without Aβ treatment showed no effect on DHE intensity ([Fig. 2](#)). To verify this technique of measurement for superoxide anions, we demonstrated that NADPH oxidase inhibitor (gp91 ds-tat Peptide 2) suppressed Aβ-mediated increase in DHE intensity. The inhibitor alone had no effect on DHE intensity. In sum, Aβ-induced ROS production in astrocytes through NADPH oxidase activation was suppressed by laser irradiation.

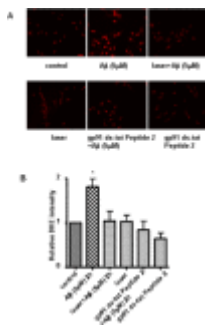


Fig. 2

Laser (632.8nm) suppressed Aβ-induced ROS production in astrocytes

Laser light suppressed Aβ-induced assembly of NADPH oxidase subunits in primary astrocytes

Since ROS production through NADPH oxidase is preceded by translocation of cytosolic subunits to bind with membrane subunits, we tested the effects of laser on colocalization between cytosolic p47^{phox} and membrane-associated gp91^{phox} after treating astrocytes with Aβ. Confocal immunofluorescence microscopy of gp91^{phox} and p47^{phox} subunits showed that stimulation of astrocytes by Aβ for 2 h led to a significant increase in colocalization between these two subunits, while laser pretreatment suppressed the colocalization to the control level (Fig. 3). These results suggest that laser attenuates Aβ-induced NADPH oxidase activation by inhibiting the assembly of its subunits. Astrocytes exposed to laser without Aβ treatment showed no effect on colocalization (Fig. 3). To validate the fluorescent confocal microscopy method for measurement of the colocalization between these two subunits, we demonstrated that NADPH oxidase inhibitor (gp91 ds-tat Peptide 2) suppressed Aβ-mediated increase in colocalization (Fig. 3). The inhibitor alone had no effect on the colocalization. These data suggest that laser attenuates Aβ-induced ROS production through inhibiting NADPH oxidase activation.

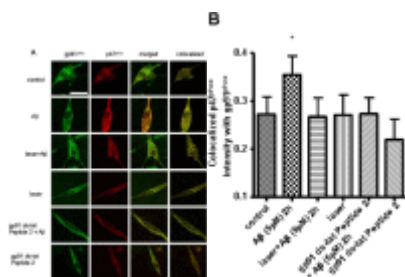


Fig. 3

Laser (632.8nm) suppressed Aβ-induced colocalization between NADPH oxidase subunits gp91^{phox} and p47^{phox} in astrocytes

To rule out the possibility that laser and Aβ might have affected the expressions of p47^{phox} and gp91^{phox}, which may also cause changes in the colocalization between p47^{phox} and gp91^{phox}, Western blot analysis showed that laser and Aβ treatments did not change the total expressions of p47^{phox} and gp91^{phox} in astrocytes (Fig. 4).

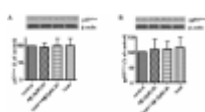
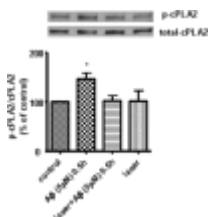


Fig. 4

Laser (632.8nm) and Aβ did not alter the expression of p47^{phox} and gp91^{phox} in astrocytes

Laser suppressed Aβ-induced phosphorylation of cPLA₂ in primary astrocytes

ROS from NADPH oxidase can stimulate downstream signaling pathways including activation of MAPK which further leads to phosphorylation of cPLA₂ (Shelat et al., 2008). p-cPLA₂ targets mitochondria and triggers further ROS production from mitochondria (Zhu et al., 2006). Since our data suggested that laser suppressed A β -induced ROS production through inhibiting NADPH oxidase activation, we hypothesized that laser also suppresses A β -induced phosphorylation of cPLA₂. In support of our hypothesis, Western blot analysis of p-cPLA₂ and cPLA₂ showed that stimulation of astrocytes by A β for 30 min led to a significant increase in phosphorylation of cPLA₂, while laser pretreatment suppressed the phosphorylation to the control level (Fig. 5). Astrocytes exposed to laser without A β treatment exerted no effect on phosphorylation of cPLA₂ (Fig. 5).

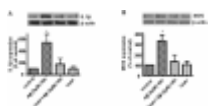


[Fig. 5](#)

Laser (632.8nm) suppressed A β -induced phosphorylation of cPLA₂ in astrocytes

Laser suppressed A β -induced expression of pro-inflammatory factors in primary astrocytes

AD is associated with increased inflammatory responses (Griffin et al., 1998), such as increase in IL-1 β (Zhu and Qian, 2006). Since there is evidence that low-level light can offer anti-inflammatory effects (Sakurai et al., 2000, Freitas et al., 2001, Lim et al., 2007, Aimbire et al., 2008, Boschi et al., 2008, Hammer et al., 2008), we tested whether laser was capable of suppressing A β -induced inflammation in astrocytes. Western blot analyses of IL-1 β and iNOS showed that stimulation of astrocytes by A β for 16 h led to a significant increase in the expressions of IL-1 β and iNOS, while laser pretreatment suppressed the expressions to the control level (Fig. 6). Astrocytes exposed to laser without A β treatment showed that laser itself had no effect on expressions of these inflammatory factors (Fig. 6).



[Fig. 6](#)

Laser (632.8nm) suppressed A β -induced expressions of IL-1 β and iNOS in astrocytes

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DISCUSSION

The present study demonstrated, for the first time, the ability of laser light at $\lambda = 632.8$ nm to suppress A β -induced ROS production and inflammatory response in primary rat astrocytes. Specifically, we demonstrated the ability of this laser to suppress A β -induced ROS production, colocalization between NADPH oxidase subunits gp91^{phox} and p47^{phox}, phosphorylation of cPLA₂, and expression of pro-inflammatory factors including IL-1 β and iNOS.

AD is a prominent neurodegenerative disease affecting a large proportion of the aging population (Hebert et al., 2003). Although oxidative stress has been implicated in the progression of this disease, the mechanism for production of ROS has not been clearly elucidated (Gupta et al., 1991, Smith et al., 1991, Behl et al., 1994, Mecocci et al., 1994, Yan et al., 1994, Cini and

[Moretti, 1995](#), [Forster et al., 1996](#), [Simonian and Coyle, 1996](#), [Smith et al., 1996](#), [Mecocci et al., 1997](#), [Behl and Holsboer, 1998](#), [Schippling et al., 2000](#), [Hamilton et al., 2001](#), [Lovell and Markesbery, 2007](#)). Our previous studies have shown that A β triggered NADPH oxidase activation to induce ROS and subsequently phosphorylated cPLA₂ in primary astrocytes ([Zhu et al., 2006](#)). Furthermore, cPLA₂ was shown to cause a decrease in mitochondrial membrane potential ($\Delta\Psi_m$) and result in more ROS production ([Zhu et al., 2006](#)). Interestingly, low-level laser ($\lambda=635\text{nm}$) has been reported to increase $\Delta\Psi_m$ and ATP synthesis ([Bortoletto et al., 2004](#)). Another study showed that low-level infrared laser protected PC12 cells against oxidative stress through its ability to modulate $\Delta\Psi_m$ ([Giuliani et al., 2009](#)). In fact, low-level laser therapy has been reported to prevent oxidative stress in rat traumatized Achilles tendon ([Fillipin et al., 2005](#)) and Duchenne muscular dystrophy patients ([Abdel et al., 2007](#)). Our data showed that 632.8 nm laser suppressed A β -induced ROS production ([Fig. 1](#)) through inhibiting colocalization of NADPH oxidase subunits, gp91^{phox} and p47^{phox} ([Fig. 2](#)). It is known that the interactions of gp91^{phox} with the cytosolic components result in a conformational change in gp91^{phox}, which enables the electron flow from NADPH to oxygen and the generation of superoxide anions ([Han et al., 1998](#), [Diebold and Bokoch, 2001](#)). In this study, our results lead to a new hypothesis that low-energy laser light may be capable of causing a conformation change in gp91^{phox}, which inhibits the interaction of gp91^{phox} with the cytosolic components and the generation of superoxide anions. More investigations are needed to further our understanding in the mechanism of inhibiting the assembling of gp91^{phox} with the cytosolic components by low-energy laser light. Consistent with previous studies reporting that phosphorylation of cPLA₂ is one of the downstream pathways of NADPH oxidase ([Zhu et al., 2006](#), [Shelat et al., 2008](#)), our data also showed that A β -induced p-cPLA₂ was suppressed by laser pretreatment ([Fig. 3](#)). Since cPLA₂ is associated with perturbation of mitochondria $\Delta\Psi_m$, results from this study suggested that laser pretreatment reduced ROS from NADPH oxidase and subsequently ROS-induced mitochondrial dysfunction through cPLA₂.

Inflammation is an early event of AD and plays critical roles in disease development ([Tuppo and Arias, 2005](#), [Wyss-Coray, 2006](#), [Cameron and Landreth, 2010](#), [McNaull et al., 2010](#)). The major players involved in the inflammatory process in AD are microglia and astrocytes ([McGeer and McGeer, 1995, 2001, Combs, 2009](#)). In AD, A β plaques are surrounded by activated microglia and astrocytes ([Wallace et al., 1997](#), [Yin et al., 2006](#), [Bolmont et al., 2008](#), [Koenigsknecht-Talboo et al., 2008](#), [Yan et al., 2009](#)) which have been shown to secrete many pro-inflammatory molecules, such as cytokines, chemokines, prostaglandins, complement proteins and complement inhibitors ([McGeer and McGeer, 1995, 2001, Zhang et al., 2009](#)). Among these pro-inflammatory molecules, IL-1 β is an important regulator of inflammatory cascades and plays a key role in AD pathogenesis ([Griffin and Mrak, 2002](#)). IL-1 β is capable of stimulating astrocytes to produce additional pro-inflammatory cytokines such as IL-6, another inflammation marker associated with neurodegeneration ([Blom et al., 1997](#), [Fiebich et al., 1998](#), [Griffin et al., 1998](#)). Furthermore, IL-1 β triggers activation of NF κ B ([Cao et al., 1996](#), [Song et al., 1997](#), [Baeuerle, 1998](#)) which is necessary for A β -stimulated iNOS induction in astrocytes ([Akama et al., 1998](#), [Akama and Van Eldik, 2000](#)). iNOS is responsible for production of nitric oxide ([Lee et al., 1993](#), [Weldon et al., 1997](#)) which may be detrimental to neurons ([Hu et al., 1997](#)). Low-level laser attenuates inflammation ([Sakurai et al., 2000](#), [Freitas et al., 2001](#), [Lim et al., 2007](#), [Aimbire et al., 2008](#), [Boschi et al., 2008](#), [Hammer et al., 2008](#)) and helps tissue repair and wound healing ([Whelan et al., 2001](#), [Whelan et al., 2003](#), [Albertini et al., 2007](#), [Correa et al., 2007](#), [Viegas et al., 2007](#), [Reis et al., 2008](#)). However, the effects of laser on A β -induced inflammation in astrocytes have not been studied. Here we showed that laser pretreatment suppressed A β -induced the expression of IL-1 β in astrocytes ([Fig. 4A](#)). Consistent with the notion that A β stimulation of

iNOS in astrocytes is IL-1 β dependent ([Akama and Van Eldik, 2000](#)), laser also attenuated iNOS induction in astrocytes ([Fig. 4B](#)). Since IL-1 β is a key player in AD pathogenesis and iNOS activity has detrimental effects on neurons, 632.8 nm laser may also protect neurons against inflammation in AD brain.

Previous studies suggest that low-energy light exerts beneficial effects through modulating gene expressions ([Shefer et al., 2002](#), [Eells et al., 2004](#)). Brief treatment with 670 nm light-emitting diode (LED) regulates expression of genes encoding DNA repair protein, antioxidant enzymes and molecular chaperons in retinas of methanol intoxicated rats ([Eells et al., 2004](#)). Low-energy laser irradiation (632.8nm, 4.5mW, 3 s) also increases the expression of anti-apoptotic protein Bcl-2 and reduces the expression of pro-apoptotic protein Bax in muscle cell cultures ([Shefer et al., 2002](#)).

There has been a large body of compelling evidence suggesting that biological effects of lights could be used to treat neurodegenerative conditions, although the mechanism has yet to be fully understood. *In vitro*, LED attenuates apoptosis in PC12 cells after exposure to A β_{25-35} ([Duan et al., 2003](#)). LED also increases survival and ATP content of neurons and decreases oxidative stress after rotenone-induced toxicity ([Liang et al., 2008](#)). In fact, LED also promotes neurite outgrowth of rat cortex in tissue culture ([Wollman and Rochkind, 1998](#)). *In vivo*, near-infrared light exerts neuroprotective effects against rotenone-induced neurotoxicity in rats ([Rojas et al., 2008](#)), and these effects was supported by assessing behavioral, morphological and neurochemical changes ([Rojas et al., 2008](#)). LED has been shown to induce central and peripheral nerve regeneration after trauma ([Anders et al., 1993](#), [Byrnes et al., 2005](#)) and reduce neuroinflammation in rats ([Byrnes et al., 2005](#)). In this study, we demonstrated that 632.8 nm laser suppressed A β -induced ROS production and inflammation in primary astrocytes. Although we did not investigate the dose response by varying irradiance (W/cm²) and energy density (J/cm²), it is important to note that a biphasic dose response has been demonstrated many times in low level laser therapy research ([Huang et al., 2009](#)). These dose dependence studies applied irradiance ranging from 0.7 to 40 mW/cm² and energy density ranging from 0.18 to 9 J/cm². While the irradiance (1.5 mW/cm²) was applied in this study, the energy density (16.2 J/cm²) was outside the range of those in previous studies from others. Therefore, one might consider optimizing the use of light by applying various irradiance, and exposure time. Another parameter for optimization is the wavelength, because a longer wavelength is known for a deeper tissue penetration. For example, 810 nm LED light enhances mitochondrial metabolism ([Trimmer et al., 2009](#)), and transcranial infrared laser (805 nm) therapy has been reported to improve clinical rating scores after embolic strokes in rabbits ([Lapchak et al., 2004](#)). This study serves as a proof-of-principle to provide insights into potential applications of laser therapy for attenuating oxidative and inflammatory responses in AD.

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Abbreviations

AD Alzheimer's disease

A β amyloid β -peptide

BCA bicinchoninic acid

BSA Bovine serum albumin

cPLA₂ cytosolic phospholipase A₂

DHE dihydroethidium

DMEM Dulbecco's modified Eagle's medium

DMSO dimethyl sulfoxide

FBS fetal bovine serum

HFIP hexafluoro-2-propanol

IL-1 β interleukin-1 β

iNOS inducible nitric-oxide synthase

LED light-emitting diode

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Footnotes

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