Cellular Model Studies of Brain-mediated Phototherapy on Alzheimer's Disease

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ABSTRACT

Alzheimer's disease (AD) is now the most common neurodegenerative disease. Despite approval of several drugs for AD, the disease continues to rob millions of their memories and their lives. We have studied the cellular models of brain-mediated phototherapy on AD, and the studies will be reviewed in this paper. Genetic studies have shown that dysfunction of amyloid β-protein (Aβ) or tau is sufficient to cause AD. Aβ or Aβ induced redox stress induced neuron apoptosis might be as a cellular model of AD. We found red light at 640±15 nm from light emitting diode array (RLED640) might inhibit Aβ 25-35 induced PC12 cell apoptosis, which is mediated by cyclic adenosine monophosphate, and it might inhibit hydrogen peroxide (H₂O₂) induced differentiated PC12 cell (dPC12) apoptosis, which is mediated by tyrosine hydroxylase. There is rhythm dysfunction in AD. We found low intensity 810 nm laser irradiation might rehabilitate TNF-alpha induced inhibition of clock gen expression of NIH 3T3 fibroblasts. Our studies provide a foundation for photobiomodulation on brain to rehabilitate AD.

KEYWORDS: Alzheimer's disease (AD), Photobiomodulation

Alzheimer’s disease (AD), the most common cause of dementia in the elderly, with more than 20 million cases worldwide, is characterized by progressive and insidious neurodegeneration of the central nervous system leading to a gradual decline of cognitive function and dementia [1]. The major neuropathological features of AD are synaptic and neuronal degeneration and the presence of amyloid plaques and neurofibrillary tangles (NFTs) [2]. There is a century since the first AD description in 1907 by Alois Alzheimer [3]. Over the past 25 years, it has become clear that the proteins forming the deposits are central to the disease process. Two hypothetical models are proposed, in which beta-amyloid (Aβ) and tau represent the key element. Aβ and tau make up the amyloid plaques and NFTs of AD, where these normally soluble proteins assemble into amyloid-like filaments. Genetic studies have shown that dysfunction of Aβ or tau is sufficient to cause dementia [4]. Despite approval of several drugs for AD, the disease continues to rob millions of their memories and their lives. The lack of treatments with a major impact might be discouraging. Fortunately, basic research is identifying many of the pathways that contribute to this devastating disease, providing unprecedented opportunities for

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the development of new treatments aimed at the root causes of AD\textsuperscript{[5]}. The predicted increase in AD cases over the next few decades makes the development of better treatments a matter of utmost importance and urgency\textsuperscript{[5]}.

Photobiomodulation (PBM) is a modulation of laser irradiation or monochromatic light (LI) on biosystems, which stimulates or inhibits biological functions but does not result in irreducible damage\textsuperscript{[6]}. The LI used in PBM is always low intensity LI (LIL), \textasciitilde 10 mW/cm\textsuperscript{2}, and its PBM was denoted as LPBM. The LPBM on cells provide a foundation for its therapeutic applications\textsuperscript{[7]}. Nick Lane\textsuperscript{[8]}, a science writer based in London, recently explored that phototherapy relieve not just acute toxicity, but more chronic inflammatory conditions, and shed therapeutic light on the implications for cancer and degenerative diseases. Xu \textit{et al.}\textsuperscript{[9]} have treated AD patients with intranasal He-Ne laser irradiation at 3.5\textasciitilde4.5 mW for 30 min each time, which was done once every morning for 30 days, and found that melatonin, score in mini-mental state exam and score in Wechsler memory scale for adult increased. Lampl \textit{et al.}\textsuperscript{[10]} have used 808 nm infrared laser to irradiate the shaved head of the patient with ischemic stroke at about 1 J/cm\textsuperscript{2}, and found the infrared laser therapy has shown initial safety and effectiveness for the treatment of ischemic stroke in humans. We have studied the cellular models of brain-mediated LPBM on AD, and the studies will be reviewed in this paper.

1. AMYLOID PROTEIN 25-35 INDUCED APOPTOSIS IN PC12

A\textsubscript{β} is the major components of senile plaques in the brain of the AD patients\textsuperscript{[11-13]}, its aggregation and AD course development closely related, and may induce the neurons in vitro to show the characteristics of apoptosis\textsuperscript{[14]}. Now, more and more researches suggested that AD neurodegeneration is A\textsubscript{β} induced apoptosis in nature\textsuperscript{[11-13, 15]}. As the direct anti-apoptotic is an important strategy of AD pharmacological treatment\textsuperscript{[16]}, our laboratory devoted in developing the skull exposure to light to inhibit neuronal apoptosis as a possible way of AD phototherapy. In the previous study\textsuperscript{[17]}, we adopted A\textsubscript{β}25-35 induced apoptosis of rat pheochromocytoma cells (PC12 cells) as the cell model of AD phototherapy preliminary study, and then used the red light emitter diode (640\textpm15 nm) (RLED640) to study its PBM. It was found that RLED640 at 0.09 mW/cm\textsuperscript{2} for 60 min has significantly diminished A\textsubscript{β}25-35 induced apoptosis of PC12 cells. Further research\textsuperscript{[18]} found such light treatment had no effects on PC12 cell proliferation, but promoted the enhancement of intracellular Cyclic Adenosine Monophosphate (cAMP) level and to cause PC12 cell secrete anti-apoptosis factors. A\textsubscript{β}25-35 incubation can enhance intracellular CAMP level, and RLED640 promoted further the enhancement. Zhang L \textit{et al.}\textsuperscript{[19]} also found low intensity He-Ne laser irradiation at 0.52 mW/cm\textsuperscript{2} for 5\textasciitilde40 min also inhibited A\textsubscript{β}25-35 induced PC12 cell apoptosis, and its fluorescence resonance energy transfer (FRET) imaging indicated that the inhibition was mediated by PKC.

2. HYDROGEN PEROXIDE-INDUCED APOPTOSIS IN DIFFERENTIATED PC12

AD is a multifactorial entity with a complex pathogeny. As the pathogenesis of this disorder is not known, various hypotheses have been developed based on experimental data accumulated. An increasing number of studies showed that oxidation reactions occur in AD and that A\textsubscript{β} may be one molecular link between oxidative stress and AD-associated neuronal cell death\textsuperscript{[20-23]}. Consequently, antioxidant approaches for the prevention and therapy of AD are of central interest. One of the plausible ways to prevent the reactive oxygen species (ROS)-mediated cellular injury is dietary or
pharmaceutical augmentation of endogenous antioxidant defense capacity. In our study, we have investigated the effects of PBM of RLED640 on H$_2$O$_2$-induced oxidative stress and apoptotic death in differentiated PC12 (dPC12) cells. Oxidative stress has been considered as a major cause of cellular injuries. DPC12 cells were subjected to hydrogen peroxide (H$_2$O$_2$) at 0-300 µmol/L or/and RLED640 at 0.03 or 0.06 mW/cm$^2$ for 10, 20 or 60 min, respectively. The irradiation was done in three ways, daily (from the 1$^{st}$ to 3$^{rd}$ day), every other day (the 1$^{st}$, 3$^{rd}$ day) and on only the 1$^{st}$ day from the oxidative stress induction day on. The results showed that RLED640 attenuated H$_2$O$_2$-induced ATP change ($P <0.01$), apoptosis change ($P <0.05$) and DNA fragmentation ($P <0.01$) for at least more than 24 hours. Among the different dosages and the three irradiation ways, the irradiation with RLED640 at 0.06 mW/cm$^2$ for 10 min every other day had the highest cell viability ($P <0.01$). After dPC12 cells were subjected to 150 µmol/L H$_2$O$_2$ and RLED640 at 0.06 mW/cm$^2$ for 20 min, the tyrosine hydroxylase (TH) mRNA level and the level of caspase-3 expression increased and decreased in irradiation group ($P <0.01$), respectively. In conclusion, PBM of RLED640 has protective effect on dPC12 cells against oxidative stress induced by H$_2$O$_2$.

3. TUMOR NECROSIS FACTOR INDUCED CLOCK GENE EXPRESSION INHIBITION IN NIH3T3 FIBROBLASTS

Dementia in AD patients is associated with circadian rhythm disturbances, probably because of Aβ-induced neuronal damage of the central pacemaker located in the hypothalamic suprachiasmatic nuclei (SCN) [25, 26]. Preliminary data suggest that light treatment might improve the circadian activity rhythms deteriorate of AD patients [26]. Campbell et al. [27] found that 3 h of bright light exposure to the area behind the knee caused phase shifts of the human circadian rhythms, but Wright et al. [28] did not find the circadian phase resetting. In Campbell et al.’s experiment, the ambient light less than 20 lux might disturb the sleep homeostasis so that there was PBM on the popliteal region and then on circadian rhythms through meridian. However, the participants in Wright et al.’s experiment were shielded from ocular light (0 lux) during extraocular light exposure so that the participants might be in sleep homeostasis and no PBM was found.

Campbell et al.’s experiment was supported by our cellular model [29]. The expression of the circadian clock genes in NIH3T3 fibroblasts was synchronized by 50% horse serum shock [30]. Its suppression was induced by TNF-alpha at 10 ng/mL final concentration. Its PBM was then done with low intensity 810 nm laser irradiation (LIDL) at dose [intensity (mW/cm$^2$) × irradiation time (mins)], dose 1, 5×5; dose 2, 5×10; dose 3, 10×5; dose 4, 10×10; dose 5, 10×20. LIDL at dose 1 and dose 5 promoted and inhibited the TNF-alpha induced expression suppression (TAES) of mPer1, mPer2, mPer3 and mDbp ($P<0.01$). LIDL at dose 2 inhibited TAES of mPer1 ($P<0.01$). LIDL at dose 3 inhibited the TAES of mPer1 and mDbp ($P<0.01$). LIDL at dose 4 inhibited the TAES of mPer1, mPer3 and mDbp ($P<0.01$). Among doses 2-5, LIDL at dose 4 was the most effective in inhibiting the TAES. The results showed that TNF-alpha induced inhibition effect of the circadian clock genes’ expression may be modulated with LIDL. Further studies are needed to assess PBM on circadian clock disorder in AD patients.

4 DISCUSSION

Our cellular model studies support the therapeutic effects of brain-mediated LPBM on AD. Its safety will be studied
in this section.

LPBM is a safe modality for clinical use\textsuperscript{[31, 32]}. Complete laser hatching of human embryos using the Zona infrared laser optical system does not have an adverse effect on subsequent development\textsuperscript{[33]}. There is no cytotoxic and genotoxic potential of LIL (660 nm, 12 mW, 5 kHz, 2 and 20 J/cm\textsuperscript{2}) on mammalian cells\textsuperscript{[32]}. The induction of cell-cycle delay of visible-light irradiation at 660 nm is not initiated by deoxyribonucleic acid (DNA) strand breaks\textsuperscript{[34]}. Following the doses of infrared A (IRA) (700-2000 nm) that induced ferritin levels, there was no alteration seen for nuclear DNA type damage, oxidative stress proteins or proteases involved in the degradation of skin\textsuperscript{[35]}. The difference in the frequency of micronuclei between pre- and post-laser irradiation indicates that a LHNL at such energy densities 1, 2, 3 and 5 J/cm\textsuperscript{2} does not induce micronucleus formation\textsuperscript{[36]}.

Drug-induced liver injury (DILI) or drug-induced liver dysfunction might be one of the side effects of the use or chronic use of drugs\textsuperscript{[37]}. However, there are no side effects of LPBM on liver. DILI is a major health problem that challenges not only health care professionals but also the pharmaceutical industry and drug regulatory agencies. According to the United States Acute Liver Failure Study Group, DILI accounts for more than 50\% of acute liver failure, including hepatotoxicity caused by overdose of acetaminophen (39\%) and idiosyncratic liver injury triggered by other drugs (13\%). Because of the significant patient morbidity and mortality associated with DILI, FDA has removed several drugs from the market, including bromfenac, ebrotidine, and troglitazone. Other hepatotoxic drugs, such as risperidone, trovafloxacin, and nefazodone, have been assigned “black box” warnings. DILI is the most common cause for the withdrawal of drugs from the pharmaceutical market. DILI is initiated by direct hepatotoxic effects of a drug, or a reactive metabolite of a drug.

Drug-induced kidney injury is a major side effect in clinical practice\textsuperscript{[38]}. However, there are no side effects of LPBM on kidney. Renal injury associated with drugs may involve several components of the kidney: glomerulus, tubules, interstitium and blood vessels. Acute renal failure may occur as a major reaction to many drugs. Moreover, therapeutic agents may induce an allergic reaction leading to interstitial inflammation and tubular damage\textsuperscript{[39]}. Although the exact incidence of drug-induced nephrotoxicity is not known, it is important for clinicians to be aware of the risks in certain patients and to know which drugs are the most commonly implicated. The latter include radiocontrast agents, aminoglycosides, nonsteroidal anti-inflammatory drugs, and angiotensin-converting enzyme inhibitors. Other medications also have nephrotoxic potential when they are prescribed in specific patient populations. Renal injury may be transient and mild in many cases, but recognition of the patient at high risk and application of preventive measures are essential to avoid a severe and protracted course\textsuperscript{[38]}.

As LI photons have no rest mass, there are no metabolite problems in LPBM and then there are no side effects of LPBM on liver or kidney. There have not been any side effects found in its applications in health care, health promotion or disease treatment. Moreover, there are no effects of LIL on biosystem in homeostasis. Therefore, LPBM should be a natural therapy and can be used as long term therapeutic approach on the chronic AD.

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