Free Radical Biology and Medicine 53 (2012) 1459–1467

Contents lists available at SciVerse ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

Photobiomodulation by low-power laser irradiation attenuates Aβ-induced cell apoptosis through the Akt/GSK3β/β-catenin pathway

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ARTICLE INFO

Article history:
Received 16 January 2012
Received in revised form
5 July 2012
Accepted 2 August 2012
Available online 11 August 2012

Keywords:
Apoptosis
Alzheimer disease
Amyloid β peptide
GSK3β
β-Catenin
Akt
Low-power laser irradiation
Free radicals

ABSTRACT

Apoptosis induced by amyloid β peptide (Aβ) is thought to associate with the pathogenesis of Alzheimer disease (AD). Accumulating evidence shows that low-power laser irradiation (LPLI) is capable of reducing Aβ-induced apoptosis. However, the underlying mechanisms remain unclear. In this study, we report a novel molecular mechanism by which LPLI attenuates $A\beta_{25-35}$-induced apoptosis through the Akt/GSK3β/β-catenin pathway. We found that Akt activated by LPLI interacted with GSK3β and phosphorylated it on Ser9 in the presence of $A\beta_{25-35}$, which resulted in the inhibition of GSK3β. Furthermore, LPLI increased the nuclear translocation of β-catenin and enhanced its T cell factor/lymphocyte enhancer factor-dependent transcriptional activity via the Akt/GSK3β pathway to promote cell survival upon treatment with $A\beta_{25-35}$. Our data demonstrate that LPLI has a prosurvival effect on Aβ-induced apoptosis and may be an effective therapeutic strategy in treating AD by targeting GSK3β.

Introduction

Alzheimer disease (AD) is a chronic neurodegenerative disease that is characterized by a progressive decline in multiple cognitive functions, resulting in memory loss and dementia. Extracellular buildup of amyloid β peptide (Aβ) aggregates, which results in the formation of senile plaques, represents one of the neuropathological hallmarks of AD, and considerable efforts have been expended in understanding the relationship of Aβ to AD [1,2]. Previous studies demonstrate that apoptosis caused by Aβ is implicated in the pathogenesis of AD [3–6]. Various signaling pathways have been reported to account for the mechanisms of Aβ-induced apoptosis thus far [4,7–11]. In addition, there have been studies indicating that proteins involved in Aβ-induced apoptosis can be potential therapeutic targets for the treatment of AD [12,13]. However, further studies are needed to illustrate the mechanisms of AD and search for effective therapeutic strategies.

It is known that glycogen synthase kinase 3β (GSK3β) is a proapoptotic gene playing important roles in Aβ-induced neuronal apoptosis [14–18]. GSK3β drives the formation of intracellular neurofibrillary tangles (NFTs), which is one of the hallmarks of AD, by promoting hyperphosphorylation of the tau protein [19,20]. Moreover, β-catenin, as an integral component in the canonical Wnt signaling pathway, is involved in controlling the development of the vertebrate central nervous system [21,22]. In the Wnt pathway, the unphosphorylated β-catenin is stable and translocates into nuclei to promote cell survival by activating its T cell factor/lymphocyte enhancer factor (TCF/LEF)-dependent transcriptional activity. GSK3β can phosphorylate β-catenin, facilitating its proteolysis to promote the neuronal degeneration in AD [14,23–25]. Together, these studies suggest that GSK3β is a promising therapeutic target in treating AD, and a therapeutic strategy targeting GSK3β is highly warranted.

Low-power laser irradiation (LPLI), as a relatively noninvasive technique that enhances both cell survival and proliferation [26–29], has been used to accelerate wound healing [30] and reduce inflammation in a variety of pathologies [31–33]. In recent years, accumulating research has suggested that LPLI could inhibit apoptosis induced by Aβ [34–36]. However, direct evidence has been lacking so far, and the underlying molecular mechanisms are not well understood. Our previous studies demonstrated that Akt was activated by LPLI to prevent staurosporine (STS)-induced apoptosis [37]. However, whether Akt is activated by LPLI in the presence of Aβ and how to exert its prosurvival function are unclear.

Abbreviations: Aβ, amyloid-β-peptide; AD, Alzheimer disease; LPLI, low-power laser irradiation; TCF, T-cell factor; LEF, lymphocyte enhancer factor; YAP, Yes-associated protein; GSK3β, glycogen synthase kinase 3β; NFTs, neurofibrillary tangles; YFP, yellow fluorescence protein; GFP, green fluorescence protein; PMSF, Phenylmethylsulfonyl fluoride; Co-IP, co-immunoprecipitation; FACS, flow cytometry assay; PI, propidium iodide; FITC, fluorescein isothiocyanate; LRP5/6, LDL receptor-related protein type 5 and 6; STS, staurosporine.

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http://dx.doi.org/10.1016/j.freeradbiomed.2012.08.003

In this study, based on various techniques and approaches, we found that LPLI suppressed GSK3β activity by activating Akt to inhibit Aβ-induced apoptosis. We further investigated the role of β-catenin during LPLI-mediated cell protection against Aβ. We found that LPLI promoted the nuclear translocation of β-catenin and increased its TCF/LEF-dependent transcriptional activity through the Akt/GSK3β pathway in the presence of $A\beta_{25-35}$. Taken together, we show, for the first time to our knowledge, that LPLI attenuated Aβ-induced apoptosis through the Akt/GSK3β/β-catenin pathway. Our data further demonstrate that LPLI has potential therapeutic value in treating AD by targeting GSK3β.

Materials and methods

Chemicals

Dulbecco's modified Eagle's medium (DMEM) was purchased from GIBCO (Grand Island, NY, USA). $A\beta_{25-35}$, Hoechst 33258, and D-luciferin sodium salt were purchased from Sigma Chemical Co. (St. Louis, MO, USA). LiCl was purchased from BioMol Research Laboratories (Plymouth, PA, USA) and API-2 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-phospho-Akt (Thr308) antibody, anti-phospho-Akt (Ser473) antibody, anti-phospho-GSK3β (Ser9) antibody, anti-Akt antibody, anti-GSK3β antibody, and anti-β-catenin antibody were acquired from Cell Signaling Technology (Beverly, MA, USA). Anti-β-actin antibody and anti-histone antibody were acquired from Santa Cruz Biotechnology. Lipofectamine reagent was purchased from Invitrogen (Carlsbad, CA, USA). $A\beta_{25-35}$ stock solution of 1 mM was prepared in distilled and deionized water and stored at −20 °C. Before a treatment, peptides were preincubated at 37 °C for 5 days to promote aggregation and then diluted with medium to desired concentrations. The concentrations of $A\beta_{25-35}$, LiCl, and API-2 used in our experiments were 25 μM, 10 mM, and 2 μM, respectively. pYFP-GSK3β was kindly supplied by Dr. John H. Kehrl [38], pGFP-β-catenin was kindly supplied by Dr. Angela I.M. Barth [39], and pTOPflash was kindly supplied by Dr. Vito Quaranta [40]. Negative control short hairpin RNA (NC shRNA) and shRNA–β-catenin were synthesized by GenePharma Co., Ltd. (Shanghai, China).

Cell culture and transfection

PC12 cells were cultured in a humidified (5% CO_2, 37 °C) incubator in DMEM (Life Technologies), supplemented with 15% fetal bovine serum, 5% horse serum, penicillin (100 U/ml), and streptomycin (100 mg/ml). To obtain neuronally differentiated PC12 cultures, subconfluent cells were differentiated for up to 5 days in DMEM with 15% fetal bovine serum and 100 nM 2.5 s NGF (Sigma–Aldrich, St. Louis, MO, USA). HEK 293T cells and SH-SY5Y cells were cultured in a humidified (5% CO_2, 37 °C) incubator in DMEM (Life Technologies), supplemented with 10% fetal bovine serum. The medium was refreshed every other day, and the cells were plated at appropriate densities according to experimental protocol. The plasmids YFP-GSK3β, GFP-β-catenin, and TOPflash were transfected into PC12, SH-SY5Y, or HEK 293T cells using Lipofectamine 2000.

Low-power laser irradiation

All groups of cells cultured for 24 h were treated with various chemicals and/or irradiated with a He–Ne laser (632.8 nm, 10 mW, 12.74 mW/cm^2; HN-1000, Guangzhou, China) at a fluence of 2 J/cm^2. The chemicals were added to the culture medium 30 min before LPLI treatment. The entire procedure was carried out at room temperature. Throughout each experiment, the cells were kept in either a complete dark or a very dim environment, except when subjected to the light irradiation, to minimize the ambient light interference.

Cell apoptosis assay

For analysis of apoptosis by nuclear staining, cell apoptosis was morphologically evaluated with Hoechst 33258. SH-SY5Y cells were treated as indicated and incubated for 6 h in 35-mm dishes. Hoechst 33258 (10 μg/ml) was added to each dish and the cells were incubated at 37 °C with 5% CO_2 for an additional 30 min in the dark. Fluorescence images of the normal and apoptotic cells were examined with a modified commercial microscope system equipped with a mercury lamp (band-pass filter 352–461 nm), a 395 nm dichroic mirror, and a long-pass filter 397 nm emission filter (LSM510/ConfoCor2; Zeiss, Jena, Germany). The fluorescence images were collected via a Zeiss C-Apochromat objective (40 ×, NA 1.3).

Quantification of apoptosis by annexin V/PI staining was performed as described previously [13]. Apoptotic cell death was determined using the BD ApoAlert annexin V–FITC apoptosis kit (Becton–Dickinson Biosciences, Mountain View, CA, USA) according to the manufacturer's instructions. Flow cytometry was performed on a BD FACSCanto II flow cytometer (Becton–Dickinson).

Time-lapse confocal fluorescence microscopy

Green fluorescent protein (GFP) and yellow fluorescent protein (YFP) fluorescence was monitored confocally using a commercial laser scanning microscope (LSM 510/ConfoCor 2) combination system (Zeiss) equipped with a Plan-Neofluar 40 ×/1.3 NA oil DIC objective. Excitation wavelength and detection filter settings for each of the fluorescent indicators were as follows: GFP fluorescence was excited at 488 nm with an argon ion laser and emission was recorded through a 500–520 nm band-pass filter. YFP fluorescence was excited at 514 nm with an argon ion laser and emission was recorded through a 535–545 nm band-pass filter. For time-lapse imaging, culture dishes were mounted onto the microscope stage, which was equipped with a temperature-controlled chamber (Zeiss). During control experiments, bleaching of the probe was negligible.

Western blot analysis and coimmunoprecipitation (Co-IP)

For Western blot assay, the cells were plated in 6-cm petri dishes. Briefly, the treated or untreated cells were harvested, washed with ice-cold phosphate-buffered saline (PBS), pH 7.4, and lysed with ice-cold lysis buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 1% Triton X-100, and 100 μg/ml phenylmethylsulfonyl fluoride (PMSF)) for 30 min on ice. The lysates were centrifuged at 12,000 rpm for 5 min at 4 °C, and the protein concentration was determined. Equivalent samples (40–100 μg of protein extract was loaded in each lane) were subjected to SDS–PAGE on 10 or 15% gels. The proteins were then transferred onto polyvinylidene difluoride membranes and probed with the indicated antibodies, followed by secondary antibodies: goat anti-mouse conjugated to Alexa Fluor 800 or goat anti-rabbit conjugated to IRDyeTM 680. Detection was performed using the Li-Cor Odyssey infrared imaging system (Li-Cor, Lincoln, NE, USA). Cells were extracted in lysis buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 1% Triton X-100, 100 μg/ml PMSF) supplemented with protease inhibitor cocktail set I for 1 h on ice. After centrifugation, the supernatant was incubated with the indicated antibody at ambient temperature for 2 h and subsequently with