Biomedicine & Pharmacotherapy 105 (2018) 813–823

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Incensole acetate prevents beta-amyloid-induced neurotoxicity in human olfactory bulb neural stem cells

Mohammed A. El-Magd[a,*], Sara F. Khalifa[b], Faisal Abdulrahman A. Alzahrani[c], Abdelnaser A. Badawy[d], Eman S. El-Shetry[e], Lamess M. Dawood[f], Mohammed M. Alruwaili[g], Hedib A. Alrawaili[h], Engi F. Risha[i], Fathy M. El-Taweel[b], Hany E. Marei[j]

[a] Department of Anatomy, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt
[b] Department of Chemistry, Faculty of Science, Damietta University, Egypt
[c] Department of Biological Sciences, Rabigh College of Science and Arts, King Abdulaziz University (Jeddah), Rabigh Branch, Rabigh 21911, Saudi Arabia
[d] Department of Medical Biochemistry, Faculty of Medicine, Mansoura University, Egypt
[e] Department of Human Anatomy, Faculty of Medicine, Zagazig University, Egypt
[f] Department of Biochemistry, Faculty of Medicine, Tanta University, Egypt
[g] Medical Laboratory Technology Department, Faculty of Applied Medical Biosciences, Northern Border University, Arar City, Saudi Arabia
[h] Medical Laboratory Sciences Department, School of Health Sciences, Quinnipiac University, Hamden, CT, USA
[i] Department of Clinical Pathology, Faculty of Veterinary Medicine, Mansoura University, Egypt
[j] Department of Cytology and Histology, Faculty of Veterinary Medicine, Mansoura University, Egypt

ARTICLE INFO

Keywords:
Incensole acetate
Beta amyloid
Neurotoxicity
Olfactory bulb
Neural stem cells

ABSTRACT

β-Amyloid peptide (Aβ) is a potent neurotoxic protein associated with Alzheimer's disease (AD) which causes oxidative damage to neurons. Incensole acetate (IA) is a major constituent of *Boswellia carterii* resin, which has anti-inflammatory and protective properties against damage of a large verity of neural subtypes. However, this neuroprotective effect was not studied on human olfactory bulb neural stem cells (hOBNSCs). Herein, we evaluated this effect and studied the underlying mechanisms. Exposure to $A\beta_{25-35}$ (5 and 10 μM for 24 h) inhibited proliferation (revealed by downregulation of *Nestin* and *Sox2* gene expression), and induced differentiation (marked by increased expression of the immature neuronal marker *Map2* and the astrocyte marker *Gfap*) of hOBNSCs. However, pre-treatment with IA (100 μM for 4 h) stimulated proliferation and differentiation of neuronal, rather than astrocyte, markers. Moreover, IA pretreatment significantly decreased the $A\beta_{25-35}$-induced viability loss, apoptotic rate (revealed by decreased caspase 3 activity and protein expression, down-regulated expression of Bax, caspase 8, cyto c, caspase3, and upregulated expression of Bcl2 mRNAs and proteins, in addition to elevated mitochondrial membrane potential and lowered intracellular Ca^{+2}). IA reduced Aβ-mediated ROS production (revealed by decreased intracellular ROS and MDA level, and increased SOD, CAT, and GPX contents), and inhibited Aβ-induced inflammation (marked by down-regulated expression of *IL1b, TNFa, NfKb,* and *Cox2* genes). IA also significantly upregulated mRNA and protein expression of Erk1/2 and Nrf2. Notably, IA increased the antioxidant enzyme heme oxygenase-1 (HO-1) expression and this effect was reversed by HO-1 inhibitor zinc protoporphyrin (ZnPP) leading to reduction of the neuroprotective effect of IA against Aβ-induced neurotoxicity. These findings clearly show the ability of IA to initiate proliferation and differentiation of neuronal progenitors in hOBNSCs and induce HO-1 expression, thereby protecting the hOBNSCs cells from $A\beta_{25-35}$-induced oxidative cell death. Thus, IA may be applicable as a potential preventive agent for AD by its effect on hOBNSCs and could also be used as an adjuvant to hOBNSCs in cellular therapy of neurodegenerative diseases.

Abbreviations: Aβ, β-Amyloid peptide; AD, Alzheimer's disease; HO-1, heme oxygenase-1; hOBNSCs, human olfactory bulb neural stem cells; IA, incensole acetate; iPSCs, induced pluripotent stem cells; MMP, mitochondrial membrane potential; MSCs, mesenchymal stem cells; Nes, nestin; ZnPP, zinc protoporphyrin

* Corresponding authors.
E-mail address: mohamed.abouelmagd@vet.kfs.edu.eg (M.A. El-Magd).

https://doi.org/10.1016/j.biopha.2018.06.014
Received 6 April 2018; Received in revised form 2 June 2018; Accepted 3 June 2018

1. Introduction

β-Amyloid peptide (Aβ) is a potent neurotoxic protein which together with intracellular neurofibrillary tangles constitute the main hallmark for Alzheimer's disease (AD) [1,2]. Aggregation of this peptide induces oxidative stress [1,3] which subsequently leads to neural apoptosis in AD [4,5]. The $A\beta_{25–35}$ peptide, which recently detected in elderly people, was reported to have more potent neurotoxic effects than the well characteristic full length amyloid peptide, $A\beta_{1–42}$ [6–8].

Drug treatments and cells based therapies are the most promising strategy for treatment of neurodegenerative diseases. Several types of stem cells, including embryonic stem cells, neural stem cells (NSCs), human olfactory bulb neural stem cells (hOBNSCs), induced pluripotent stem cells (iPSCs), and mesenchymal stem cells (MSCs) have been investigated as a cellular therapy for neural injuries and associated neurodegenerative diseases. Among these cells, hOBNSCs therapy is more acceptable for ethical, moral, and safety concerns. Our previous experiments showed the potential of hOBNSCs to restore cognitive deficit in AD and to regenerate neural damage in spinal cord of rats [9–11]. On the other side, controversial results were obtained regarding the therapeutic effect of other stem cells. Some studies showed negative effect for these cells on AD progression and this was attributed to presence of unfavorable constituents, such as Aβ, which inhibit NSCs proliferation and survival [12–16]. In contrast, other reports showed either a stimulatory effect of Aβ on NSCs proliferation, suggesting enhanced neurogenesis [2,17,18], or no significant effect [19]. None of these studies was applied on hOBNSCs.

AD is associated with oxidative stress and inflammation. Many studies highlighted natural herbs as potential neuroprotective candidates to attenuate oxidative stress induced by Aβ deposition in neural cells [7,20]. Boswellic acid derived from *Boswellia* species was widely used for treatment of various types of inflammation and injuries [21] and as memory enhancer [22]. Incensole acetate (IA), a main constituent of *Boswellia carterii* resin, has neuroprotective effects against neuronal damage in traumatic and ischemic head injury [23,24]. IA exerts its anti-inflammatory effect through inhibition of pro-inflammatory cytokines, including TNFa, IL1b, TGFb, Cox2, and NFkb [23–25]. However, the mechanisms by which IA mediates its effects are unclear and to the best of our knowledge, no publications are available in the literature that address Aβ-induced cytotoxicity in hOBNSCs.

Additionally, ample previous experimental data indicate positive role played by the antioxidant enzyme heme oxygenase-1 (HO-1) in protecting neurons against oxidative stress damage induced by Aβ [6,7]. HO-1 protein levels are normally low in neurons, however it can be remarkably increased during the formation of Aβ in AD brain [26]. Several studies have reported that Erk1/2 can activate cytosolic Nrf2 and help its translocation to nucleus (to protect it from cytosolic degradation) where it promotes synthesis of HO-1 [reviewerd in 7]. Thus, in the present study, we aimed to elucidate the effect of IA on hOBNSCs proliferation and differentiation and to investigate the underlying mechanisms regulating IA effects on Aβ-induced neurotoxicity with special concern to the role of HO-1 and its upstream modulators, Erk1/2 and Nrf2.

2. Materials and methods

2.1. Human OBNSCs culture and treatment

The olfactory bulbs (OBs) were obtained from two adult patients undergoing craniotomy at the Institute of Neurosurgery, Catholic University, Rome, Italy [27]. Prior signed agreements were obtained according to protocols approved by the Ethical Committee of the Catholic University. Obtained OBs were directly broken down at 37 °C in 0.1% Papain (Sigma-Aldrich, St. Louis, MO, USA) for 30 min. Cell suspensions were grown in a proliferation medium (DF12) composed of DMEM/F12 (1:1) (Invitrogen), 0.025 mg/mL progesterone, 2 mM L-glutamine, 0.6% glucose, 5.2 ng/mL sodium selenite, 0.025 mg/mL insulin, 0.1 mg/mL apo-transferrin sodium salt, 9.6 μg/mL putrescine, 3 mM sodium bicarbonate, 5 mM Hepes, 4 mg/mL BSA, heparin 4 μg/mL (all from Sigma), 20 ng/mL epidermal growth factor (EGF; PeproTech, Rocky Hill, NJ, USA), 10 ng/mL basic fibroblast growth factor (bFGF; PeproTech, Rocky Hill, NJ, USA), and 20 ng/mL leukemia inhibitory factor (LIF; Immunological Sciences, Rome, Italy). The cells grew in form of free-floating neurospheres and were passaged twice by chemical dissociation (by incubation with accutase for 4 min at 37 °C) every 3–4 d. At passage 3 (P3), the cells were plated (single cell/mini-well) onto 96-well plates for 7 days and the formed secondary neurosphere were dissociated and then cultured (1000 cells/cm^2) in serum-free medium containing EGF and bFGF, and passaged up to P14. At P15, cells were plated (18,000 cells/cm^2) on 15 μg/ml poly-L-lysine into coated glass coverslips and cultured in DF12 medium containing EGF and bFGF for 3 d. Finally, cells grew in DF12 without growth factors (differentiation medium) for longer culture periods to induce neural differentiation.

To detect the effects of $A\beta_{25–35}$ on proliferation and differentiation of hOBNSCs, cells cultured in either proliferation or differentiation media were incubated with 5 μM [28], or 10 μM [2] of $A\beta_{25–35}$ peptide (Bachem Bioscience,Torrance, CA) for 24 h. The $A\beta_{25–35}$ peptide was prepared by incubating freshly solubilized peptides at 400 μM in sterile distilled water at 37 °C for 3 d to induce aggregation and stored frozen at −20 °C until use. To study the effects of IA, cells were pretreated with 100 μM IA for 4 h, and then $A\beta_{25–35}$ was added to the medium for 24 h. IA was extracted from *Boswellia carterii* resin and then purified and identified as previously described [29,30]. The IA dose was chosen based on a preliminary dose/response experiment using five doses, 12.5, 25, 50, 100, 200 μM, and the best neuroprotective dose, 100 μM, was selected. Parallel wells were cultured in DF12 without $A\beta_{25–35}$ or IA (untreated control group).

2.2. Detection of cell viability by MTT assay

MTT assay was performed on hOBNSCs neurospheres cultured in proliferation medium (DF12) in 96-well plates. A volume of 20 μL MTT stock solution (5 mg/ml) was added to either untreated cells, cells treated with $A\beta_{25–35}$ (at concentrations of 5 or 10 μM) alone or pretreated with IA. Absorbance of extracted resultant MTT formazan, with 150 μL dimethyl sulfoxide (DMSO), was recorded at 570 nm.

2.3. Detection of oxidative stress, mitochondrial membrane potential, and intracellular Ca^{2+}

Intracellular reactive oxygen species (ROS) was measured using the fluorescent probe 2,7-dichlorofluorescein diacetate and mitochondrial membrane potential (MMP) was detected using the rhodamine 123 (Rh123) fluorescence as previously described [7]. The levels of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) were measured using commercial kits and as previously described [20,31]. Intracytoplasmic level of Ca^{2+} was measured using imaging of the fluorescent probe Fura-2/AM as published procedure [28].

2.4. Caspase3 activity assay

Cells were homogenized in phosphate buffer saline (PBS), the lysates were centrifuged at 15,000 rpm for 10 min at 4 °C and supernatants were collected. Protein concentration was determined by Bradford assay. Equal amounts of protein were incubated for 1 h at 37 °C with the specific fluorogenic substrate of caspase3 (7-amino-4-methylcoumarin *N*-acetyl-L-aspartyl-L-glutamyl-L-valyl-L-aspartic acid amide). Cleavage of the caspase substrates was detected using a fluorescence microtitre plate reader at excitation/emission wavelengths of 360/460 nm.