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Neuro-protective effects of aloperine in an Alzheimer's disease cellular model

Jing Zhao[a,1], Ge Zhang[b,1], Min Li[a], Qinghua Luo[a], Yu Leng[c,**], Xu Liu[a,*]

[a] Department of Neurology, The Second Affiliated Hospital of Nanchang University, Nanchang, 330006, China
[b] Department of Emergency, The First People's Hospital of Jiujiang City, Jiujiang, 332000, China
[c] Department of Emergency, The First People's Hospital of Jiujiang City, No.48, south Taling Road, Jiujiang, Jiangxi province, 330029, China

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ABSTRACT

Excessive production of amyloid β (Aβ) induced by familial mutations in amyloid precursor protein (APP) and presenilin 1 (PS1) results in neuronal oxidative insults, mitochondrial dysfunction, and apoptosis, which play an essential role in the pathological development of Alzheimer's disease (AD). Aloperine, a quinolizidine alkaloid derived from the leaves of the Sophora plant, has displayed multiple pharmacological functions in several chronic diseases. In the current study, we investigated the neuro-protective effects of aloperine against cytotoxicity in mouse Neuro2a (N2a) cells transfected with Swedish amyloid precursor protein (Swe-APP) mutant and presenilin 1 exon 9 deletion mutant (N2a/Swe.D9). We found that aloperine ameliorated oxidative stress patterns in N2a/Swe.D9 cells by reducing the production of reactive oxygen species (ROS) and 4-hydroxy-2-nonenal (4-HNE). Additionally, aloperine treatment led to elevated generation of ATP and increased mitochondrial membrane potential (MMP) in N2a/Swe.D9 cells. Importantly, we found that aloperine treatment reduced the vulnerability of N2a/Swe.D9 cells to H_2O_2. Aloperine also inhibited apoptosis of N2a/Swe.D9 cells via a mitochondria-dependent pathway. These findings suggest that aloperine may have pharmacological potential for the treatment of AD.

1. Introduction

Amyloid-β (Aβ), a peptide with 39–42 amino acids, is one of the most causative factor in Alzheimer's disease (AD) [1]. Aβ is derived from amyloid precursor protein (APP) undergoing two sequential proteolytic processing by β- and γ-secretase. Presenilin 1 (PS1) is the catalytic core of γ-secretase [2]. Mutations in APP and PS1 have been associated with familial AD, which accounts for less than 10% of AD cases worldwide. Swedish double mutations (K670 N/M671 L) are one of the most important mutations of APP, which has been reported as a missense mutation in the brains of an AD family [3]. Several PS1 mutations including exon 9 deletion have been reported in previous studies. Overexpression of APP Swedish double mutations and PS1 exon 9 deletion in neuronal cells results in excessive production of Aβ, especially Aβ42 [4]. Excessive production and aggregation of neurotoxic forms of Aβ has been identified as playing an important role in the pathological development of AD. Increased levels of reactive oxygen species (ROS) and overproduction of lipid peroxidation have been found in APP/PS-1 double mutant neurons [5]. Mitochondrial dysfunction is an early event in the pathogenesis of AD. Decreased mitochondrial membrane potential (MMP) and intracellular ATP levels have been found in AD brains [6]. A "mitochondrial cascade hypothesis" which includes reduced energy metabolism, enhanced oxidative stress, and apoptosis has been proposed to explain the molecular mechanism in AD brains. Stress-activated protein kinases (SAPKs) such as JNK and p38 play a casual role in triggering mitochondria-dependent apoptosis and activating the release of cytochrome C from mitochondria to cytosol [7]. Blockage of Aβ-induced cytotoxicity has been considered as an important therapeutic strategy for AD treatment.

Aloperine is a novel quinolizidine alkaloid derived from the leaves and seeds of the Sophora plant [8]. A unique bridged tetracyclic ring system exists in the core of this alkaloid. The molecular structure of aloperine is shown in Fig. 1. Increasing evidence that aloperine possesses multiple pharmacological properties in several diseases,

* Corresponding author at: Department of Neurology, The Second Affiliated Hospital of Nanchang University, No.1 Minde road, Nanchang, Jiangxi province, 330006, China.
** Corresponding author.
E-mail addresses: lengyu026@163.com (Y. Leng), xuliu002@yeah.net (X. Liu).
[1] Jing Zhao and Ge Zhang are co-first authors.

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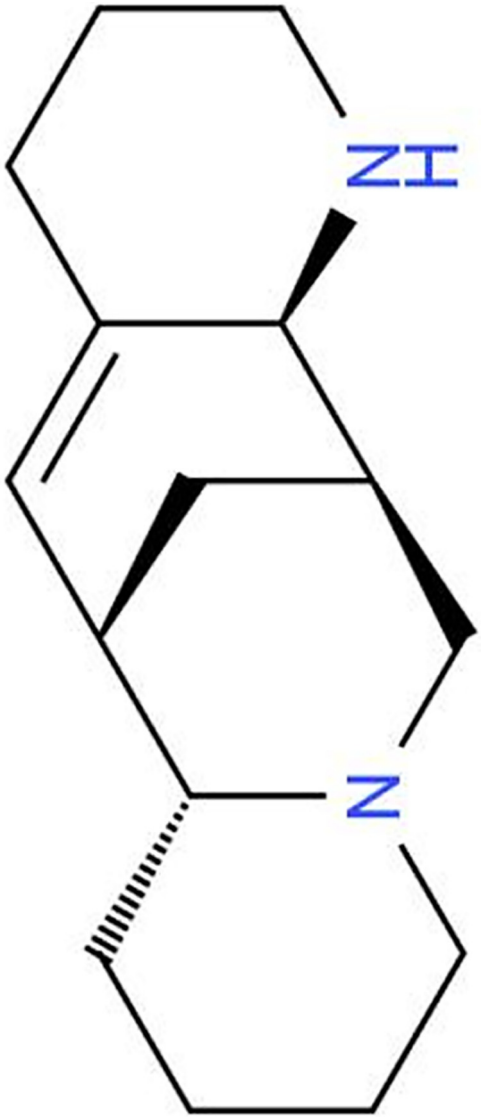

Fig. 1. Molecular structure of Aloperine.

including acute inflammation, hypersensitivity, and adjuvant arthritis, has been reported [9]. A recent study identified the anti-infection capacity of aloperine by showing that aloperine and its derivatives act as a new class of anti-HIV-1 entry inhibitors [10]. Aloperine can attenuate H_2O_2-induced apoptosis by regulating the AKT and NF-κB signaling pathways in nucleus pulposus cells [11]. Notably, administration of aloperine exerted neuroprotective effects against ischemia-reperfusion (IR)-induced acute renal injury by preventing inflammatory infiltration, inhibiting ROS generation, and suppressing tubular apoptosis [12]. However, little information regarding the effects of aloperine against Aβ-induced neurotoxicity in AD has been reported before.

2. Materials and methods

2.1. Cell culture and treatment

Neuroblastoma N2a cells stably transfected with empty vector (N2a/Control) or co-transfected with Swedish mutant APP and ΔE9 deleted presenilin-1 (N2a/Swe.D9) obtained from Dr. H.-X. Xu (The Burnham Institute, SD, USA) were used in this study. Cells were maintained in DMEM/OPTI–MEM (V/V:1:1) containing 5% FBS, 200 μg/mL G418, and 0.1% antibiotics (penicillin and streptomycin, P/S) [13]. Cells were treated with 50 and 100 μM Aloperine for 24 h. N2a/Swe.D9 cells were treated with 100 μM H_2O_2 in the presence or absence of 50 and 100 μM aloperine for 24 h.

2.2. Measurement of Aβ in conditioned culture media

N2a/Control and N2a/Swe.D9 cells were plated in 96-well plates with an appropriate density according to each experimental scale. Media were used for measurement of secreted Aβ40 and Aβ42 by sandwich ELISA. The following ELISA kits were used in this study: human Aβ40 Quantikine ELISA Kit (#DAB140B, R&D Systems, USA); human Aβ42 Quantikine ELISA Kit (#DAB142, R&D Systems, USA). Experiments were performed in accordance with the manufacturer's instructions.

2.3. Determination of cell viability

N2a/Swe.D9 cells were seeded into a 96-well cell culture plate at a density of 5×10^4 cells per well. After 12 h, N2a/Swe.D9 cells were treated with 100 μM H_2O_2 in the presence or absence of 50 and 100 μM aloperine for 24 h. Then, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) (5 mg/mL) was added to each well and incubated for 2 h in darkness. Culture medium was washed away and 100 μl dimethyl sulfoxide (DMSO) was added. After gently shaking on a shaker for 10 min, OD value was detected at 490 nm using a microtiter plate reader.

2.4. Measurement of lactate dehydrogenase (LDH) release

A commercial LDH kit was used to detect LDH release. N2a/Swe.D9 cells were seeded into a 96-well cell culture plate at a density of 5×10^4 cells per well. After 12 h, N2a/Swe.D9 cells were treated with 100 μM H_2O_2 in the presence or absence of 50 and 100 μM aloperine for 24 h. Briefly, equal amounts of cultural medium and reaction agent were mixed together and incubated at room temperature for 30 min. After stopping the reaction with 50 μl stop solution, OD value was measured at 490 nm to calculate LDH release.

2.5. Determination of intracellular glutathione (GSH) levels

N2a/Swe.D9 cells were treated with aloperine at the concentrations of 50 and 100 μM for 24 h. A 5,5′-dithiobis-2-nitrobenzoic acid–-glutathione disulfide reductase recycling assay was used to evaluate intracellular GSH levels in N2a/Swe.D9 cells [14]. Protein concentrations measured using the BCA method. The level of GSH was normalized to protein concentrations.

2.6. Measurement of glutathione peroxidases (GPx) activity

N2a/Swe.D9 cells were cultured on a 6-well plate at a density of 2×10^5/ml for 12 h. Cells were then treated with 50 and 100 μM aloperine for 24 h. Reaction buffer containing 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM GSH, 0.2 U/mL GSH reductase, 0.2 mM tert-butyl-hydroperoxide, and 0.2 mM NADPH was added and NADPH disappearance was measured at 340 nm.

2.7. Determination of reactive oxygen species (ROS)

Intracellular ROS was determined using a fluorescence probe—2′, 7′ dichlorofluorescin diacetate (DCFH-DA, Sigma-Aldrich) (Life Technologies, USA). Briefly, N2a/Control and N2a/Swe.D9 cells were seeded onto cell culture slides in 6-well plates. Cells were washed with HBSS and loaded with 10 μM DCFH-DA (Sigma-Aldrich, USA) in a CO_2 incubator for 30 min at 37 °C in darkness. Green fluorescent signals were visualized using a fluorescence microscope (#IBE2000, Zeiss, Germany) (excitation: 485 nm and emission: 538 nm) at a magnification of 20 × . Average fluorescence intensity was analyzed by Image-