Journal of Advanced Zoology

ISSN: 0253-7214
*Volume **44** Issue **S-5 Year 2023** Page **1433:1438***

Evaluation Of *Alocasia Cordfolia* Rhizomes Extract for Its Potential To Inhibit Oligomeric Amyloid β-Induced Microglial Activation Through NADPH Oxidase

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Article History

Received: 23 June 2023
Revised: 09 Sept 2023
Accepted: 27 Oct 2023

Abstract

Objective: The search for natural neuroprotective components from plants has received extensive attention worldwide. The purpose of this study was to examine the inhibitory effect of Alocasia cordfolia rhizomes extract (ACRE) on Oligomeric Amyloid β (oAβ)-induced microglial activation,and further to assess whether this effect is mediated through NADPH oxidase. Methods: Microglia activation model was established by inducing BV-2 microglia cells with oAβ. After treated with the extract for 24 h, cell viability was detected using cell counting kit-8 (CCK-8)assay, intracellular levels of ROS, MDA and SOD, as well as contents of NO, IL-1β and TNF-α incell culture media were analyzed with commercial kits. Next, the protein expression of gp91phox was determined by Western blot. Results: In oAβ-stimulated BV-2 microglia cells, cell proliferation was significantly increased, intracellular ROS and MDA levels were markedly raised while SOD activity was reduced, and contents of NO, IL-1β and TNF-α in cell culture media were distinctly elevated. Meanwhile, gp91phox protein expression was obviously increased. However, these effects could be dramaticallyreversed dose-dependently by ACRE. Conclusion: ACRE may be a potentially promising natural neuroprotective products for the treatment of AD, due to its excellent activity in inhibiting microglial proliferation and activation induced by oAβ.

Keywords: *Alocasia Cordfolia, Rhizomes, Alzheimer's Disease, Microglial Activation, NADPHOxidase*

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by cognitive impairment and progressive dementia, whose incidence and prevalence increase with age. AD, cardiovascular disease, and malignancy are among the most prevalent, disabling and costly disorders worldwide with the development of aging population [1]. Accordingly, prevention and treatment of AD has become a hot issue of global concern. AD's pathogenesis is complex, and its main neuropathological hallmark is senile plaques formed by the accumulation of amyloid-β protein (Aβ) [2]. Neuroinflammation mediated by activated microglial cells around senile plaques has been advocated as the main pathogenic cause of AD. Activated microglia can release a variety of neurotoxic factors, such as reactive oxygen species (ROS), nitric oxide (NO), interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), which can cause neuronal damage and death eventually, also active more microglia cells and form a vicious cycle, thereby leading to occurrence or progression of AD[3, 4].

Therefore, inhibiting the productionof neurotoxic factors by activated microglial cells may be a promising strategy for AD therapy. Among the various cytotoxic factors released by activated microglia, ROS has been confirmed as akey role in microglia-mediated neuroinflammation. NADPH oxidase is a superoxide-producing enzyme system that is the major source of ROS in microglia [4, 5]. Besides, NADPH oxidase- dependent production of ROS has been reported to be involved in AD. Consequently, it may be aneffective means of preventing or treating AD by regulating NADPH oxidase activity and hence ROSgeneration. *Alocasia cordfolia*, locally known in Malaysia as "birah negeri", is an herbaceous plant belonging to the Araceae family. In many countries, different parts of this plant have been used traditionally totreat cough, toothache rheumatic, typhoid, tuberculosis, diuretic, laxative, astringent, malaria, typhoid and tuberculosis [6]. *Alocasia cordfolia* is one of the most abundant natural resources in Malaysia, however, it has not been fully utilized due to too few basic studies. Several studies have reported that the leaves of *Alocasia cordfolia* have several biological effects, such as antioxidant, antifungal, antinociceptive, anti-inflammatory, antidiabetic, hypolipidemic and hepatoprotective

[7]. Yet, few researches have been conducted on the chemical constituents and pharmacological activity of *Alocasia cordfolia* rhizomes. Our previous studies found that the rhizomes of *Alocasia cordfolia* contain various bioactive ingredients including phenolics and flavonoids, thereby exhibiting good radical scavenging activity. The aim of this study is to evaluate the inhibitory effect of *Alocasia cordfolia* rhizomes extract on microglial activation induced by Oligomeric Amyloid β (oAβ) and then to investigate whether the inhibitory effect is mediated through NADPH oxidase.

Materials And Methods

Collection and authentication of plant materials

The mature plant of *Alocasia cordfolia* was purchased from Katsura Garden Center, Malaysia. Plant authentication was performed by Dr.Sreemoy Kanti Das in the Faculty of Pharmacy, Lincoln University College, where a voucher specimen was deposited (No. SBID: 001/21.

Preparation of extract of *Alocasia cordfolia* rhizomes

The rhizomes of *Alocasia cordfolia* were washed with tap water, cut into small pieces, dried first under sunlight for 3 days and placed in a hot air oven at 50°C until constant weight. Then the driedplant rhizomes were crushed by a Philips HR2221/01 blender to obtain a fine powder. The powderedrhizomes of *Alocasia cordfolia* were extracted using different solvents with increasing order of polarity (chloroform, acetone and ethanol) via a Soxhlet apparatus. The ethanol extraction solutionwas evaporated till dryness with the help of a water bath, thereby obtaining 5.6 g of *Alocasia cordfolia* rhizomes extract (ACRE). The extract was dissolved in dimethyl sulfoxide (DMSO) to a concentration of 50 mg/mL as stock, and then diluted with culture medium to the desired concentration for use in the following experiments. The final concentration of DMSO in all sampleswas not higher than 0.1%.

Preparation of oAβ

oAβ was prepared according to the literature reported [8]. Briefly, the amyloid-β 1-42 peptide (Sigma-Aldrich, USA) was first dissolved at a concentration of 1 mM in 1,1,1,3,3,3-hexafluoro-2- propanol (Aladdin Chemistry Co. Ltd, Shanghai, China), aliquoted, dried under vacuum, and storedat -80°C until use. Dried peptide was dissolved in DMSO to obtain a 5 mM concentration, which was further diluted with ultrapure water to obtain a 100 μM solution, and then was incubated at 4°Cfor 24 h. Thus, oAβ was obtained and added in the subsequent experiments with a final concentration of 2 μM.

Cell Culture and Treatments

The murine microglia cell line BV-2 was obtained from Institute of Basic Medical Sciences, ChineseAcademy of Medical Sciences (Beijing, China). Cells were cultured in high-glucose DMEM medium (KeyGen, Nanjing, China) supplemented with 10% fetal bovine serum (FBS; Zhejiang Tianhang Biotechnology Co., Ltd, Hangzhou, China), 100 U/mL penicillin and 100 μg/mL streptomycin at 37°C, in a humidified atmosphere containing 5% CO_2. The culture medium was replaced every 2–3 days until the cells reached confluence, and confluent cells were passaged withtrypsin-EDTA solution. Cells at passages 3-6 were used for the following tests.

BV-2 microglia cells in logarithmic growth phase were seeded into culture plates. After incubationfor 24 hours, cells of different groups were treated as follows: (1) the control group was incubated in only DMEM medium for another 24 hours; (2) the oAβ group was incubated in DMEM with oAβstimulation for another 24 hours; (3) the drug-treated group was pretreated with different concentrations of ACRE (50, 100, 250 μg/mL) for 2 h and then co-cultured with oAβ for another 24h.

Cell Viability Assay

Cell viability was measured using Cell Counting Kit-8 (CCK-8; KeyGen, Nanjing, China). BV-2 microglia cells were seeded into 96-well culture plates with a density of 1×10^4 cells/well. After relevant incubations, 10 μL of CCK-8 solution was added to each well of the plate and further incubated for 2 hours. Finally, the absorbance was measured at 450 nm using a microplate reader (BIO-RAD 680, USA). The cell survival rate was calculated as follows: Cell survival rate (%) = (OD experimental group/ OD control group) ×100.

Detection of ROS, MDA, SOD, NO, IL-1β and TNF-α

BV-2 microglia cells were seeded into 6-well culture plates at a density of 5×10^5 cells/well. After relevant treatments, the cell culture media was collected, centrifuged, and analyzed for the levels ofNO, TNF-α and IL-1β. Meanwhile, the cells were harvested and diluted to 1.0×10^6 cells/mL with PBS (pH 7.2-7.4). Cells then were broken up via repeated freezing and thawing to release the intracellular components and subjected to centrifugation at 12,000 rmp for 15 min. After centrifugation, the supernatant was used to detect the levels of ROS, MDA, SOD. Briefly, ROS, TNF-α and IL-1β levels were determined using Mouse ROS Elisa Kit, TNF-α Elisa Kit and IL-1β Elisa Kit (Enzyme-linked Biotechnology, Shanghai, China), while the contents of SOD,

MDA, andNO were measured via MDA assay kit, SOD assay kit and NO assay kit (Jiancheng Bioengineering Institute, Nanjing, China), according to the manufacturer's instructions.

Western blot assay

Western blot assay was conducted to detect the protein expression of gp91phox in the BV-2 microglia cells. BV-2 microglia cells were seeded into 6-well culture plates at a density of 5×10^5 cells/well. When the incubation time finished, BV-2 cells were washed with cold phosphate bufferedsaline (PBS) three times and lysed on ice in radio immunoprecipitation assay (RIPA) lysis buffer (P0013B, Beyotime, China) supplemented with 1 mM phenylmethanesulfonyl fluoride (PMSF, ST506, Beyotime, China) for 30 min. After centrifugation at 12,000 rmp for 30 min at 4°C, the protein concentration of the supernatants was measured via a BCA Protein Assay Kit (KGPBCA, KeyGen, Nanjing, China). Samples containing 40 μg of protein were heated at 90-100°C with sodium dodecylsulfonate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer for 5 minand then separated by 12% SDS-PAGE. Next, the separated proteins were transferred to polyvinylidene fluoride (PVDF) membranes (Sigma-Aldrich, USA). After, membranes were soakedwith 5% skimmed milk in TBST buffer for 1 h and then incubated at 4°C overnight with rabbit anti-gp91phox antibodies (1:1000, Abcam), followed by incubated with horseradish peroxidase (HPR)-conjugated goat anti-rabbit antibodies (1:1000, Beyotime) for 1h at room temperature. Finally, the blots were visualized using an enhanced chemiluminescence (ECL) detection kit (P0018, Beyotime,China) and quantified by Image J software. Relative protein expression levels were normalized to β-actin.

Statistical Analysis

All data are expressed as the mean ± SD and analyzed by SPSS 20.0 statistical analysis software. Comparison between two groups was analyzed by Student t test, and comparison among multiple groups were performed by one-way ANOVA. $P< 0.05$ was considered to be statistically significant.

Results And Discussion

Effect of ACRE on cell viability of BV-2 microglia cells

CCK-8 assay was first carried out to evaluate whether ACRE treatment would affect the cell viability of BV-2 microglia cells. Cells were incubated without or with ACRE at different concentrations (from 25 μg/mL up to 1000 μg/mL) for 24 h, and the results were shown in Figure. 1. The cell survival rate of BV-2 microglia cells treated with ACRE was not significantly altered atconcentrations ranging from 25 to 250 μg/mL as compared to that of control group ($P > 0.05$). However, the cell viability was significantly decreased compared with control ($P < 0.01$) when the concentration of ACRE was higher than 500 μg/mL, indicating a cytotoxic effect on BV-2 microglia cells. Therefore, concentrations of 50, 100, 250 μg/mL were used for all subsequent experiments.

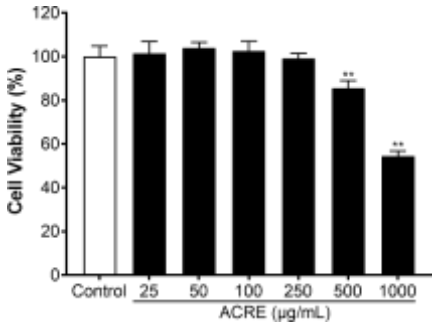

Figure 1: Effect of ACRE on cell viability of BV-2 microglia cells. BV-2 microglia cells were incubated without or with different concentrations of ACRE (25-1000 μg/mL) for 24 h, cell viabilitywas then determined with CCK-8 assay. Cell survival rate was presented as mean ± SD from five independent experiments.

$^{**}P < 0.01$ indicates a significant difference compared with the control group by one-way ANOVA.

Effect of ACRE on oAβ-induced cell proliferation in BV-2 microglia cells

Microglial proliferation is a characteristic feature in the progression of AD [9], so we investigated the inhibitory effect of ACRE on oAβ-induced cell proliferation in BV-2 microglia cells using CCK-8 assay. As shown in Figure 2, oAβ obviously promoted the BV-2 microglia cell proliferation compared to control cells ($P < 0.01$). However, compared with the oAβ group, a significant dose- dependent decrease in cell proliferation was observed after treatment with ACRE ($P < 0.05$ or $P < 0.01$). The results obtained suggested that ACRE had the potential to inhibit BV-2 microglia cellproliferation induced by oAβ.

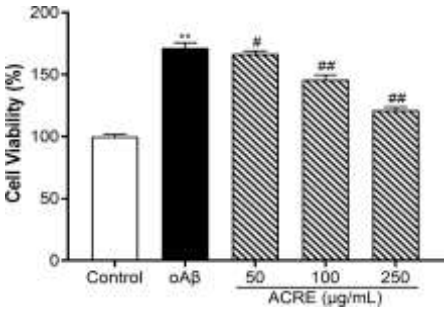

Figure 2: Effect of ACRE on oAβ-induced cell proliferation in BV-2 microglia cells. BV-2 microgliacells were pre-incubated with or without various concentrations of ACRE (50, 100, 250 μg/mL) for2 h, and were further incubated for 24 h with or without oAβ. CCK-8 was performed to detect the cell viability. Cell survival rate was reported as mean ± SD from five independent experiments. Significant differences were designated as follows: $^{**}P < 0.01$ compared with the control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the oAβ-induced group.

Effect of ACRE on oAβ-induced oxidative stress in BV-2 microglia cells

Oxidative stress originates from the overproduction of ROS is strongly involved in the pathogenesisof different neurodegenerative diseases, including AD[10]. To evaluate the effect of ACRE on oAβ-induced oxidative stress in BV-2 microglia cells, intracellular ROS level, MDA level and SOD activity were detected. As shown in Figure 3, the levels of ROS and MDA were significantly increased while SOD activity was markedly decreased in the oAβ-induced group, compared with the control group ($P < 0.01$). However, ACRE not only overtly decreased ROS and MDA productionbut also obviously raised SOD activity ($P < 0.05$ or $P < 0.01$), in a dose-dependent manner. The above results indicated that ACRE remarkably ameliorated oxidative stress in BV-2 microglia cellsinduced by oAβ.

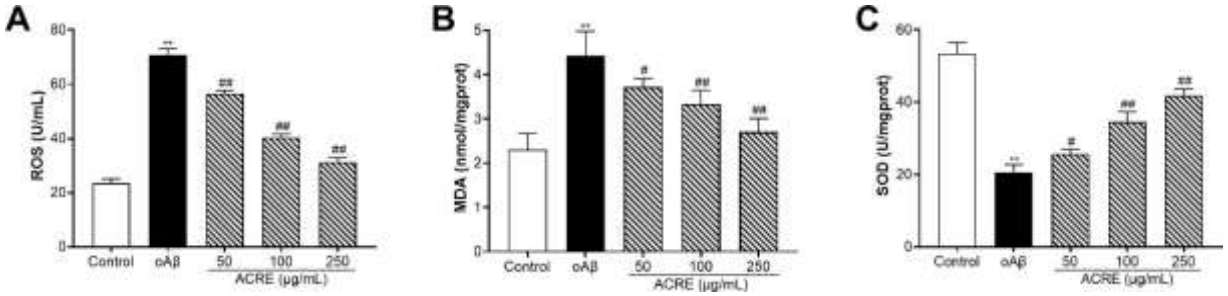

Figure 3: Effect of ACRE on oAβ-induced oxidative stress in BV-2 microglia cells. BV-2 microglia cells were pre-incubated for 2 h with or without various concentrations of ACRE (50, 100, 250μg/mL), and were further incubated with or without oAβ for 24 h. After incubation, the cell lysateswere collected and analyzed for ROS level (A), MDA level (B)and SOD activity (C). Data are shownas mean ± SD of three independent experiments. Significant differences were designated as follows: $^{**}P < 0.01$ compared with the control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the oAβ-induced group.

Effect of ACRE on oAβ-induced proinflammatory mediator release in BV-2 microglia cells

Neuroinflammation triggered by microglia has been proven to contribute to AD[11]. Consideringthat NO, IL-1β and TNF-α and are important mediators of inflammatory response, we next analyzedthe release of NO, IL-1β and TNF-α in cell culture media to assess the effect of ACRE on oAβ-induced proinflammatory mediator release in BV-2 microglia cells. As shown in Figure 4, the releaseof NO, IL-1β and TNF-α were significantly increased in oAβ-induced group ($P < 0.01$), whereas these proinflammatory mediators were significantly inhibited after ACRE treatment ($P < 0.01$). Theresults described above implied that ACRE was capable of suppressing proinflammatory mediatorrelease in BV-2 microglia cells stimulated by oAβ.

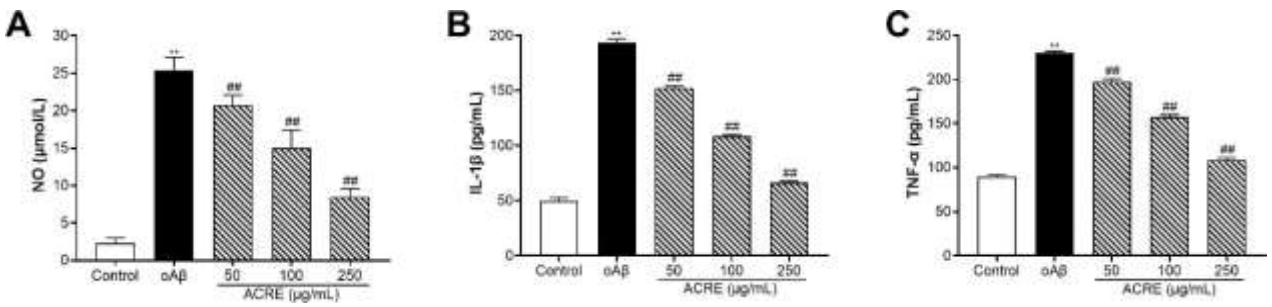

Figure 4: Effect of ACRE on oAβ-induced proinflammatory mediator release in BV-2 microglia cells.BV-2

microglia cells were pre-treated with or without various concentrations of ACRE (50, 100,250 μg/mL) for 2 h, followed by treatment with or without oAβ for 24 h. The levels ofproinflammatory mediator in cell culture media were detected. (A) NO level, (B) IL-1β level and (C) TNF-α level. Data are expressed as mean ± SD from three independent experiments. Significantdifferences were designated as follows: $^{**}P < 0.01$ compared with the control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the oAβ-induced group.

Inhibitory effect of ACRE on oAβ-induced microglia activation through NADPH oxidase

The production of neurotoxic ROS in activated microglia is mainly mediated by NADPH oxidase, and gp91phox is a predominant subunit of NADPH oxidase[4, 5]. Thus, to verify whether ACREinhibited oAβ-induced microglia activation via NADPH oxidase, the expression of gp91phox wasdetected using western blot assay. The result for gp91phox protein expression was presented inFigure 5. The protein expression of gp91phox was markedly promoted when stimulated by oAβ compared with the control ($P < 0.01$). Nevertheless, compared with the oAβ-stimulated group, gp91phox protein expression was observed to decrease in a concentration-dependent manner when incubated with ACRE ($P < 0.01$). These results demonstrated that ACRE suppressed the activationof microglia induced by oAβ through NADPH oxidase.

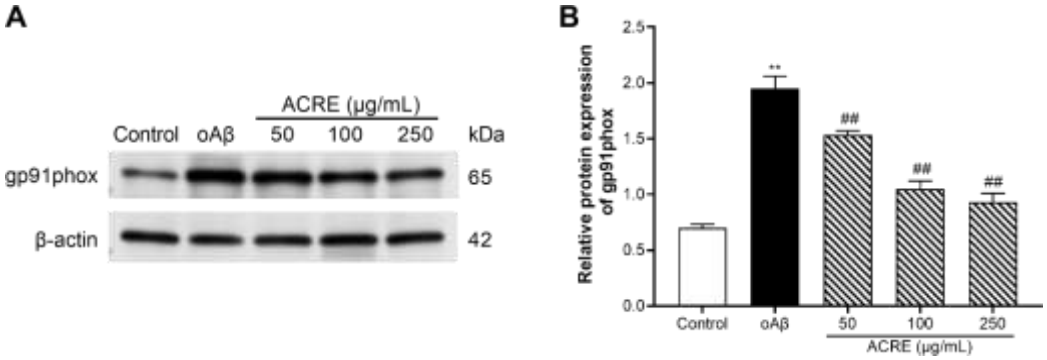

Figure 5: Inhibitory effect of ACRE on oAβ-induced microglia activation through NADPH oxidase.BV-2 microglia cells were pre-treated with or without various concentrations of ACRE (50, 100, 250 μg/mL) for 2 h, followed by treatment with or without oAβ for 24 h. The level of gp91phox protein expression was detected by western blot assay. (A) Western blot analysis and (B) relative protein expression of gp91phox. Quantification of the western blot data are described as mean ± SDfrom three independent experiments. Significant differences were designated as follows: $^{**}P < 0.01$compared with the control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the oAβ-induced group.

AD is a neurodegenerative disease, whose main pathological features are senile plaques formed bythe abnormal deposition of amyloid-β (Aβ) and neurofibrillary tangles (NFTs) formed by the hyperphosphorylation of tau protein [2]. Previous studies have reported that a large number of activated microglia around the senile plaques in the brain of AD patients and AD mice models, positively correlated with the severity of illness. Proliferation and activation of microglia are closelyassociated with the progression of AD [9]. In the early stages of AD, microglia accumulated aroundAβ plaques play a protective role by promoting clearance of Aβ. However, the early and sustained microglial proliferation promotes replicative senescence, which results in decreasing Aβ clearance and promoting Aβ accumulation. In addition, microglia undergo phenotypic specification into disease-associated microglia due to excessive proliferation, which may initiate inflammation and aggravate neuronal damage [12]. It has been demonstrated that oAβ can stimulate microglialproliferation [13]. In this study, we used oAβ to stimulate proliferation of BV-2 microglia cells, andthe results were consistent with those reported previously. Moreover, BV-2 microglia cell proliferation was significantly inhibited after ACRE treatment. These results suggested that ACREcan suppress microglial proliferation, thereby preventing or delaying the progression of AD. It is well known that neuroinflammation driven by activated microglial cells is a major pathogenic factor of AD. Microglia, the first line of defense and immune response to injuries and pathogens, are resident immune cells in the central nervous system [14]. Oxidative stress caused by the overproduction of ROS is closely related to the pathogenesis of AD, whereas microglia is regarded as the primary producer of ROS. The senile plaque deposition has been reported to active microglia,which subsequently stimulate microglial cells to produce ROS. Besides, excessive ROS can activateNF-κB, a primary regulatory target of ROS, which leads to the production of proinflammatory molecules, such as NO, IL-1β and TNF-α. These proinflammatory mediators are released continuously, which not only cause neuronal injury and ultimately contribute to neuronal death, butalso activate more microglia cells and form a vicious cycle that results in severe neuronal damage[3]. In combination, the above events eventually promote the occurrence and development of AD.

The contents of MDA and SOD in cells are representative indicators to evaluate ROS-induced oxidativestress

damage. Within the present study, oAβ can significantly increase the levels of ROS and MDA,as well as reduce SOD activity. In contrast, ACRE was able to inhibit ROS and MDA production, and enhance SOD activity in oAβ-stimulated BV-2 microglia cells. Furthermore, we found that therelease of NO, IL-1β and TNF-α in BV-2 microglia cells induced by oAβ was suppressed with the treatment of ACRE. These results proved that ACRE has a neuroprotective effect by inhibiting oxidative stress and proinflammatory mediator release in BV-2 microglia cells induced by oAβ. Related studies have demonstrated that NADPH oxidase is the main source of ROS in microglia cells. NADPH oxidase-derived ROS play an important role in microglia-mediated neuroinflammation, among various neurotoxic factors produced by activated microglia. NADPH oxidase has several family members, of which gp91phox subunit is a critical subunit that catalyzes the reduction of molecular oxygen to form ROS[4, 5]. Therefore, we examined the protein expression of gp91phox, and results showed that oAβ could promote gp91phox protein expression,whereas this effect was inhibited by ACRE treatment. Our findings, thus, indicated ACRE inhibitsoAβ-induced microglial activation via NADPH oxidase.

Conclusion

The pathogenesis of AD is quite complex, and there is a lack of effective treatments or therapeutic agents. The active ingredients from natural plants have attracted increasing attention due to their effectiveness, low toxicity and easy availability. This study suggested that ACRE has particularly good activity against AD by inhibiting microglia proliferation, ameliorating oxidative stress, and suppressing proinflammatory mediator release in oAβ-stimulated BV-2 microglia cells. Furthermore, we found that this action is mediated through downregulation of NADPH oxidase. Therefore, further studies are required to identify the specific active compounds present in this extract and to elucidate their mechanisms in AD therapy.

References

1. Falkenhain, K., Ruiz-Uribe, N. E., Haft-Javaherian, M., Ali, M., Catchers, S., Michelucci, P. E., et al. (2020). A pilot study investigating the effects of voluntary exercise on capillary stalling and cerebral blood flow in the APP/PS1 mouse model of Alzheimer's disease. PLoS One, 15(8), e0235691.
2. Hu, Q., Yu, B., Chen, Q., Wang, Y., Ling, Y., Sun, S., et al. (2018). Effect of Linguizhugan decoction on neuroinflammation and expression disorder of the amyloid β-related transporters RAGE and LRP-1 in a rat model of Alzheimer's disease. Molecular Medicine Reports, 17(1), 827-834.
3. Li, B., Dasgupta, C., Huang, L., Meng, X., & Zhang, L. (2020). MiRNA-210 induces microglial activation and regulates microglia-mediated neuroinflammation in neonatal hypoxic-ischemic encephalopathy. Cellular & Molecular Immunology, 17(9), 976-991.
4. Zhang, F., Shi, J-S., Zhou, H., Wilson, B., Hong, J-S., & Gao, H-M. (2010). Resveratrol protects dopamine neurons against lipopolysaccharide-induced neurotoxicity through its anti-inflammatory actions. Molecular Pharmacology, 78(3), 466-477.
5. Neal, M. L., Boyle, A. M., Budge, K. M., Safadi, F. F., & Richardson, J. R. (2018). The glycoprotein GPNMB attenuates astrocyte inflammatory responses through the CD44 receptor. Journal of Neuroinflammation, 15(1), 1-14.
6. Arbain, D., Sinaga, L. M. R., Taher, M., Susanti, D., Zakaria, Z. A., & Khotib, J. (2022). Traditional uses, phytochemistry, and biological activities of Alocasia Species: A systematic review. Frontiers in Pharmacology, 13, 849704.
7. Islam, M. K., Mahmud, I., Saha, S., Sarker, A. B., Mondal, H., Monjur-Al-Hossain, A., et al. (2013). Preliminary pharmacological evaluation of Alocasia indica Schott tuber. Journal of Integrative Medicine, 11(5), 343-351.
8. Shukla, S. M., & Sharma, S. K. (2011). Sinomenine inhibits microglial activation by Aβ and confers neuroprotection. Journal of Neuroinflammation, 8(1), 1-11.
9. Olmos-Alonso, A., Schetters, S. T., Sri, S., Askew, K., Mancuso, R., Vargas-Caballero, M., et al. (2016). Pharmacological targeting of CSF1R inhibits microglial proliferation and prevents the progression of Alzheimer's-like pathology. Brain, 139(3), 891-907.
10. Prucolli, L., Morroni, F., Sita, G., Hrelia, P., & Tarozzi, A. (2020). Esculetin as a bifunctional antioxidant prevents and counteracts the oxidative stress and neuronal death induced by amyloid protein in SH-SY5Y cells. Antioxidants, 9(6), 551.
11. Hopp, S. C., Lin, Y., Oakley, D., Roe, A. D., DeVos, S. L., Hanlon, D., et al. (2018). The role of microglia in processing and spreading of bioactive tau seeds in Alzheimer's disease. Journal of Neuroinflammation, 15, 1-15.
12. Hu, Y., Fryatt, G. L., Ghorbani, M., Obst, J., Menassa, D. A., Martin-Estebane, M., et al. (2021). Replicative senescence dictates the emergence of disease-associated microglia and contributes to Aβ pathology. Cell Reports, 35(10).
13. Baglietto-Vargas, D., Sánchez-Mejias, E., Navarro, V., Jimenez, S., Trujillo-Estrada, L., Gómez-Arboledas, A., et al. (2017). Dual roles of Aβ in proliferative processes in an amyloidogenic model of Alzheimer's disease. Scientific Reports, 7(1), 10085.
14. Dong, W., Embury, C. M., Lu, Y., Whitmire, S. M., Dyavarshetty, B., Gelbard, H. A., et al. (2016). The mixed-lineage kinase 3 inhibitor URMC-099 facilitates microglial amyloid-β degradation. Journal of Neuroinflammation, 13(1), 1-11.