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Research Paper

Effect of Galectin-1 on Classical Microglial Activation, Neuroinflammation, and Neuronal Damage in Rats Exposed to Amyloid-β Peptide

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ABSTRACT

Introduction: Microglia-mediated neuroinflammation is one of the most striking hallmarks of Alzheimer's disease (AD). Emerging evidence indicates that microglia can be polarized into proinflammatory M1 or anti-inflammatory M2 phenotypes, and excessive activation of M1 microglia, along with neuroinflammation, is critically associated with the pathogenesis and progression of AD. The aim of the present study was to test the hypothesis that Galectin-1 (Gal-1), a member of endogenous β-galactoside-binding lectin family, may improve AD-related neuroinflammation and that the modulation of microglial polarization plays a role in this process.

Methods: Male Sprague-Dawley rats received a single intracerebroventricular (i.c.v.) injection of amyloid-β1-42 peptide (Aβ) followed by 1, 5, or 10 μg Gal-1 for 14 days. Learning and memory abilities were estimated using the Morris water maze (MWM). Then, hippocampal samples were collected from the rats, and the expression of M1 and M2 microglial markers was measured. The levels of inflammatory cytokines and neuronal injury were also evaluated.

Results: At doses of 5 and 10 μg, Gal-1 exerted a significant effect on improving Aβ-induced learning and memory impairments in rats ($P<0.05$, vs the Aβ group). Subsequently, Gal-1 efficaciously regulated microglial polarization, as evidenced by upregulating the expression of M2 microglial markers ($P<0.01$, vs the Aβ group) while downregulating M1 microglial markers ($P<0.05$, vs the Aβ group). This modulation contributed to decreasing the secretion of proinflammatory factors ($P<0.01$, vs the Aβ group) and increasing the production of anti-inflammatory factors ($P<0.01$, vs the Aβ group), as well as attenuating neuronal damage ($P<0.01$, vs the Aβ group) mediated by Aβ.

Conclusion: Gal-1 may exert neuroprotective effects by shifting microglia from the M1 phenotype to the M2 phenotype, which is beneficial for alleviating neuroinflammation. It may be a potential agent for preventing AD-like neurodegeneration.

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Highlights

- Moderate and high, but not low, doses of galectin-1 ameliorated Aβ-induced spatial memory deficits.
- Galectin-1 promoted the M1-to-M2 microglial transition and modulated inflammatory cytokine levels in Aβ-treated rats.
- Galectin-1 attenuated Aβ-induced neuronal damage in the hippocampus.

Plain Language Summary

Alzheimer's disease (AD) is a progressive brain disorder characterized by memory impairment and neuroinflammation. Microglia, the resident immune cells of the brain, can adopt different functional states. Some states support tissue protection and repair, whereas others promote inflammation and may contribute to neuronal damage. In AD, microglia tend to adopt a pro-inflammatory state, which may exacerbate neuronal injury and accelerate cognitive decline. In this study, we investigated whether galectin-1 could protect the brain in a rat model of AD-like pathology induced by amyloid-β (Aβ), a toxic protein involved in the development of the disease. Moderate and high doses of galectin-1 improved spatial learning and memory, whereas the low dose produced no significant protective effect. Galectin-1 also promoted a shift in microglia from the pro-inflammatory M1 phenotype toward the anti-inflammatory M2 phenotype. This transition reduced pro-inflammatory cytokine levels and increased anti-inflammatory cytokine levels, thereby alleviating neuroinflammation. Furthermore, galectin-1 attenuated neuronal damage in the hippocampus, a brain region essential for learning and memory. These findings suggest that galectin-1 may help alleviate Aβ-induced cognitive impairment and neuronal damage by modulating microglial polarization and inflammatory responses. Although further studies are required to confirm its safety and effectiveness, galectin-1 may represent a potential therapeutic candidate for AD.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease characterized by extracellular β-amyloid (Aβ) deposits, neurofibrillary tangles, and progressive memory loss. Although the factors contributing to AD progression are still unknown, accumulating evidence has revealed that neuroinflammation has a prominent role in the pathogenesis of AD (Bolós et al., 2017). Microglia, the resident immune cell in the central nervous system, mediates neuroinflammation in the brain (Leng & Edison, 2021). In response to Aβ stimuli, it can rapidly transform from a normally "resting" state to an "activated" state upon exposure to Aβ (Wang et al., 2021). Interestingly, many studies have noted the neurotoxic nature of activated microglia, including neuronal degeneration, and subsequent contributions to neuronal loss (Shi et al., 2019), whereas other researchers claim that activated microglia are actually beneficial to neuronal myelin repair, and neuronal plasticity and prevent neural injury by secreting neurotrophic factors (Ennerfelt et al., 2022; Yao et al., 2017). The controversies regarding the neuroprotective and neurotoxic properties of microglia may depend on their different states of polarized activation. Microglia can be polarized into classical (M1) or alternative (M2) activation states in different physiological or pathophysiological environments (Guo et al., 2022). Classically activated M1 microglia are characterized by the production of inflammatory cytokines and reactive oxygen species that contribute to neuronal damage; in contrast, alternatively activated M2 microglia release anti-inflammatory cytokines and neurotrophins that aid in neuronal repair and regenerative processes (Guo et al., 2022). A switch in the activated microglial phenotype from alternative microglia at the beginning of AD to classical microglia at an advanced stage has been reported in AD animal models (Frigerio et al., 2019). Meanwhile, an increasing number of studies have suggested that the excessive activation of M1 microglia results in an imbalance in M1/M2 microglial polarization that promotes the development of AD (Yang et al., 2022; Du et al., 2021). Therefore, strategies modifying microglial activation and polarizing microglia into the M2 state appear to represent a reasonable alternative to attenuate neuronal damage in the brains of individuals with AD.

Galectin-1 (Gal-1) is a member of the galectin family of endogenous β-galactoside-binding lectins (Ramírez et al., 2020). Gal-1 is found in the central nervous system and expressed by neurons and nonneuronal cells (Nio-Kobayashi & Itabashi, 2021). Gal-1 has been implicated in numerous fundamental cellular processes, including cell adhesion, proliferation, and interactions with mRNA splicing factors (Nio-Kobayashi & Itabashi, 2021). Recently, an increasing number of studies have supported the hypothesis that Gal-1 is involved in neuroinflammation processes. For example, Parikh and colleagues found that Gal-1 suppresses methamphetamine-induced neuroinflammation in human brain microvascular endothelial cells (Parikh et al., 2015). Similarly, Mari et al. (2016) reported that a Gal-1 deficiency resulted in low anti-inflammatory factor expression in mice with experimental encephalomyelitis. More importantly, Li and coworkers found that Gal-1 may inhibit microglia-associated proinflammatory cytokine production (Li et al., 2020), suggesting that Gal-1 has a potential role in microglial activation. Therefore, we hypothesized that Gal-1 may improve AD pathology, restrain excess M1 microglial polarization and attenuate neuroinflammation. We first investigated the effect of exogenous Gal-1 on spatial learning and memory in a rat model of Aβ-mediated AD to assess this hypothesis. Furthermore, the effects of Gal-1 on M1/M2 microglial activation, inflammatory cytokine release and neuronal damage were measured using hippocampal samples.

Materials and Methods

Chemicals and reagents

Amyloid-β1-42 peptide (Aβ), Gal-1, and 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Cresyl violet and RIPA lysis buffer were purchased from Beyotime Biotechnology (Shanghai, China). Antibodies against Ym1, CCL2, and β-actin were purchased from Abcam (Abcam, Cambridge, UK). Interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), IL-4, and IL-10 ELISA (enzyme-linked immunosorbent assay) kits were obtained from Multisciences (Hangzhou, China). The protease inhibitor mixture, phosphatase inhibitor, BCA protein assay kit, and enhanced chemiluminescence substrate kit were obtained from Pierce Biotechnology (Rockford, IL, USA). Guide cannulae were purchased from RWD Life Science (Shenzhen, China). All other chemicals used were of the highest commercially available grade.

Animals and surgery

Male Sprague-Dawley rats (8 weeks, 200-220 g) were purchased from Weitong Lihua Experimental Animal Center (Beijing, China). Animals were housed in groups of two per cage at 22±1 ˚C with a 12-hour light-dark cycle and had free access to food and water at all times. After acclimation for 5 days, animals were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) and placed in a stereotaxic apparatus (RWD Life Science). Guide cannulae for saline, Aβ, or Gal-1 intracerebroventricular (i.c.v.) administration were placed over the right lateral ventricle (AP=1 mm, L=1.6 mm, 1 mm depth) (Dong et al., 2017). Following surgery, the rats were allowed to recover for 14 days, and 50 rats were randomly divided into 5 groups of 10 animals each: control, Aβ, Gal-1L, Gal-1M, and Gal-1H groups. The animal research protocols were reviewed and approved by the Animal Ethics Committee of Yunnan University. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Drug infusion

For the i.c.v. injection, 5 μL of the appropriate solution was placed into an internal needle connected to a PE50 polyethylene tube. After unscrewing the cap, the needle was gently inserted into the guide cannula, and the solution was slowly infused into the lateral ventricle over a period of 60 s. The injection needle remained inside the guide cannula for 1 min before being slowly removed. The cap on the guide cannula was then replaced, and the rat was returned to its home cage. All rats (except the control group) received 10 μg of aggregated Aβ only once, while control rats were administered the same volume of saline. Subsequently, rats received a single daily i.c.v. injection of Gal-1 at doses of 1 μg (Gal-1L group), 5 μg (Gal-1M group), or 10 μg (Gal-1H group), or an equivalent volume of saline (control and Aβ groups) for 14 days. The Aβ peptide was dissolved in HFIP and incubated for 1 h at room temperature to prepare aggregated Aβ. HFIP was evaporated, and the dried pellet was dissolved in sterile saline at a concentration of 2 μg/μL and then incubated at 37 ˚C for 96 h to induce aggregation. Gal-1 was dissolved in sterile saline at a series of concentrations before use.

Morris water maze (MWM)

The MWM task was carried out from the 1^{2th} day to the 15th day after the Aβ injection. This MWM procedure consisted of 3 days of learning and memory training followed by a probe trial on day 4, similar to our previously

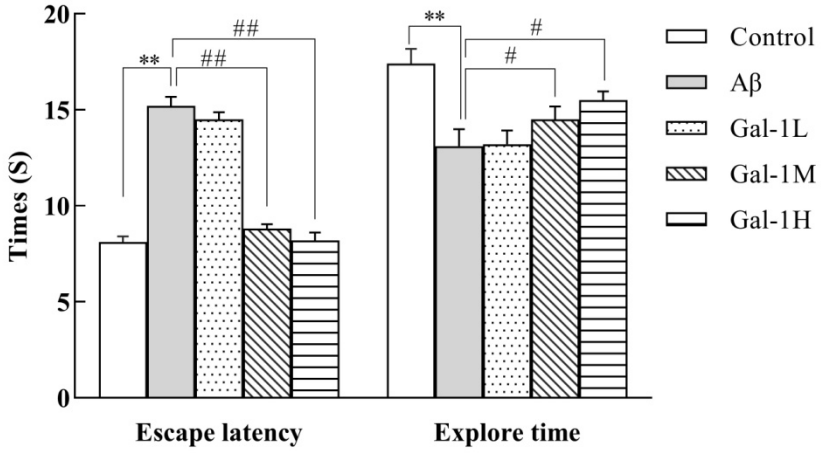

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Figure 1. Escape latency and exploration time during the probe test session of the MWM experiment

Abbreviations: Aβ: Amyloid-β1-42 peptide (10 μg); Gal-1L: Amyloid-β1-42 peptide (10 μg) + galectin-1 (1 μg); Gal-1M: Amyloid-β1-42 peptide (10 μg) + galectin-1 (5 μg); Gal-1H: Amyloid-β1-42 peptide (10 μg) + galectin-1 (10 μg).

**P<0.01 compared to the control group; #P<0.05, ##P<0.01 compared to the Aβ group.

Note: Gal-1 treatment ameliorated cognitive deficits induced by Aβ.

described method (Dong et al., 2013). Briefly, animals were trained in a circular tank located in a lit room with visual cues. The tank was 180 cm in diameter and 50 cm deep, filled with nontoxic black-dyed water maintained at 21±1 ˚C. A black escape platform (10 cm in diameter) was submerged 1 cm beneath the water surface and fixed in the center of the southwest quadrant. In each trial, each rat was randomly placed in water at one of the starting points (the midpoints of the four quadrants in the tank except the platform quadrant) and was allowed a maximum of 60 seconds to find the platform. If the animal failed to find the platform within this time, the experimenter guided the rat to the platform and allowed it to remain there for 15 seconds; the escape latency was recorded as 60 seconds. Two training trials were conducted each day for 3 consecutive days. Twenty-four hours after the last training trial, the escape latency (time to reach the platform) was recorded during the probe trial. Then, the platform was removed, and each rat was allowed to swim freely for 60 seconds inside the pool. The time spent in the southwest quadrant (exploration time) was recorded using a computerized video system (Beijing Sunny Instruments Co. Ltd., China).

Sample preparation

One day after the last behavioral test, 7 animals in each group were deeply anesthetized by isoflurane inhalation (0.5 mL/min) and decapitated. The brain was removed and dissected on ice, and hippocampi were collected for further analyses. The remaining 3 animals were perfused with phosphate-buffered saline (PBS) and 4% paraformaldehyde under deep anesthesia. Their brains were removed and fixed with 4% paraformaldehyde for 8 h at 4 ˚C prior to placing in PBS containing 10%, 20%, and finally 30% sucrose. After the brains had sunk in the final sucrose solution, 6 μm thick frozen sections were cut using a cryostat microtome (Leica, Germany) for morphological analyses.

Nissl staining

Nissl staining was done as described by Prisco with minor modification (Prisco et al., 2022). Briefly, brain sections were dried and soaked directly in chloroform for 15 min at room temperature. After washing with PBS, the sections were stained with a warm 0.05% cresyl violet solution for 20 s, rinsed with distilled water, and dehydrated in a series of graded ethanol solutions (70%, 90% and 100%). Issue slices were cleared with xylene and then cover-slipped using mounting medium. Images were captured using an optical microscope (Leica, Germany). The integral optical density of the hippocampal CA1 region was estimated using Image-Pro Plus version 6.0 (Media Cybernetics Inc., Rockville, MD, USA) to evaluate neuronal damage. Twelve sections were randomly selected for analysis, and the mean optical density was recorded for each group.

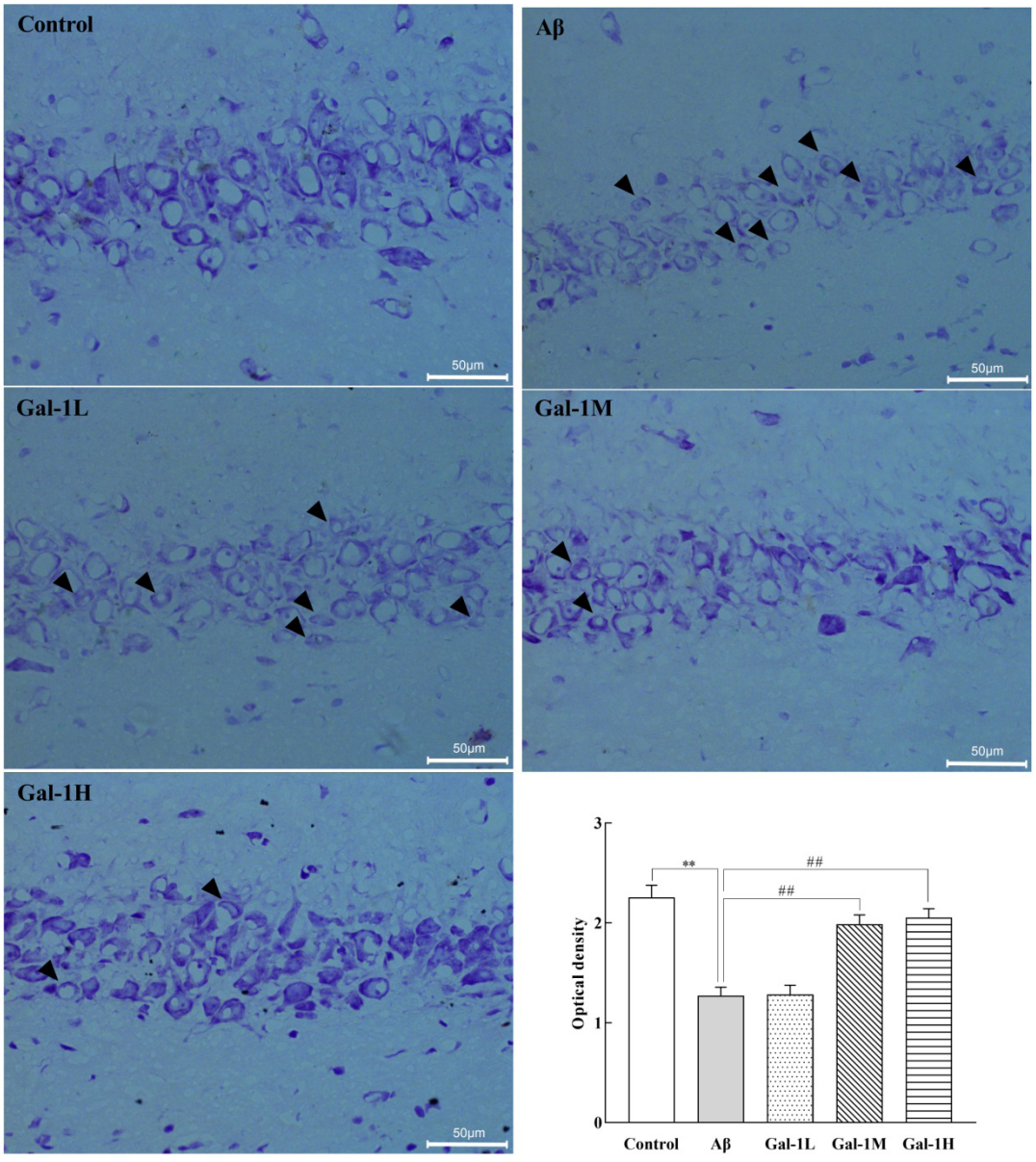

Figure 2. Neuronal injury in the hippocampal CA1 region was observed using Nissl staining NEUROSCIENCE

Abbreviations: Aβ: Amyloid-β1-42 peptide (10 μg); Gal-1L: Amyloid-β1-42 peptide (10 μg) + galectin-1 (1 μg); Gal-1M: Amyloid-β1-42 peptide (10 μg) + galectin-1 (5 μg); Gal-1H: Amyloid-β1-42 peptide (10 μg) + galectin-1 (10 μg).

**P<0.01 compared to the control group, ##P<0.01 compared to the Aβ group. Arrows indicate neuronal shrinkage.

Note: Gal-1 treatment attenuated cell damage, as shown by the increase in Nissl bodies in the cytoplasm and decreased neuronal shrinkage (×400).

ELISA

Hippocampal tissue (30 mg) was homogenized with a sonic dismembrator (ThermoFisher Scientific., Waltham, MA, USA) in 500 μL of deionized water and centrifuged at 10,000 rpm at 4 ˚C for 20 min. The supernatant was collected, and protein concentrations were determined using a BCA Protein Assay Kit. The concentrations of IL-1β, TNF-α, IL-4, and IL-10 in the supernatant were measured using ELISAs according to the manufacturer's protocol. Data are presented as pg per milligram of protein.

Western blotting

As previously described (Dong et al., 2017), hippocampal tissue was lysed with ice-cold RIPA buffer supple-

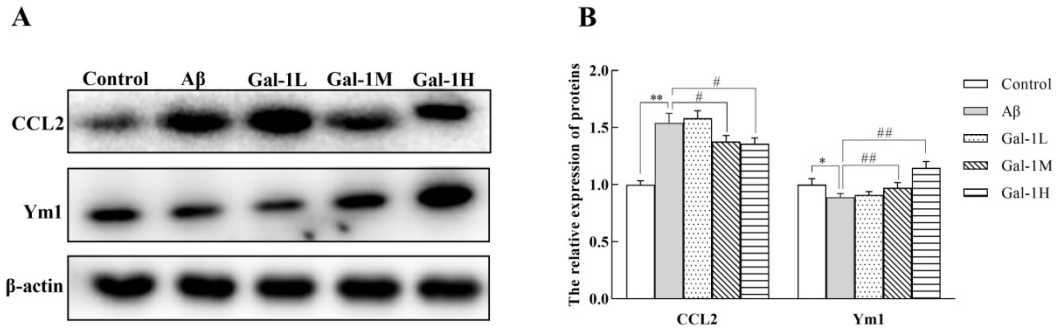

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Figure 3. Effects of Gal-1 on microglia polarization induced by Aβ

Abbreviations: Aβ: Amyloid-β1-42 peptide (10 μg); Gal-1L: Amyloid-β1-42 peptide (10 μg) + galectin-1 (1 μg); Gal-1M: Amyloid-β1-42 peptide (10 μg) + galectin-1 (5 μg); Gal-1H: Amyloid-β1-42 peptide (10 μg) + galectin-1 (10 μg).

*P<0.05 and **P<0.01 compared to the control group. #P<0.05 and ##P<0.01 compared to the Aβ group.

Note: Gal-1 treatment attenuated the upregulation of CCL2 and the downregulation of Ym1 induced by Aβ. A. M1/M2 microglial marker proteins were assessed using western blotting. B. Quantitative analysis of protein levels using densitometry. Western blot data were normalized by setting the value of the control group to 1.

mented with a protease inhibitor cocktail and centrifuged at 13,000 rpm for 10 min at 4 ˚C. The supernatant was collected, and protein concentrations were determined using a BCA Protein Assay Kit. Proteins (20 μg) were loaded and separated on SDS–PAGE gels, then transferred to PVDF membranes. Blots were blocked with 5% nonfat milk for 2 h at room temperature and then incubated overnight at 4 ˚C with the following primary antibodies: anti-Ym1 (1:1000), anti-CCL2 (1:1000), and anti-β-actin (1:2000). After washing, the membranes were incubated with a goat horseradish peroxidase-conjugated secondary antibody (1:5000) at room temperature for 1 h, followed by detection using an enhanced chemiluminescence detection kit. The band density was quantified using Image-Pro software.

Statistical analysis

Data are presented as the Mean±SEM. Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test with SPSS software, version 18.0. P<0.05 were considered statistically significant.

Results

Effect of Gal-1 on learning and memory in AD rats

As shown in Figure 1, animals that received Aβ alone showed a significantly increased escape latency but decreased exploration time in the probe test compared to control animals (P<0.01). Although 1 μg of Gal-1 had no effect on the learning and memory impairment induced by Aβ, treatment with 5 and 10 μg of Gal-1 significantly shortened the escape latency (both P<0.01, vs Aβ group) and lengthened the exploration time (both P<0.05, vs the Aβ group). However, no significant differences were observed between the Gal-1M and Gal-1H groups.

Effect of Gal-1 on hippocampal neuronal injury in AD rats

We conducted Nissl staining to investigate neuronal injury in the hippocampus. As shown in Figure 2, the results revealed pathological changes in hippocampal neurons of AD rats, such as decreased numbers of Nissl bodies, neuronal shrinkage, and even disappearance in the hippocampal CA1 region, while these phenomena were alleviated by Gal-1 (5 and 10 μg) treatment compared to the Aβ group. These observations were further supported by statistical analysis, which indicated that the mean optical density in the hippocampal CA1 region decreased in the Aβ group compared to the control group (P<0.01), and Gal-1 significantly reversed the Aβ-induced changes.

Effect of Gal-1 on microglial polarization in AD rats

We assessed the expression of the M1 marker CCL2 and the M2 marker Ym1 in the hippocampus to determine whether microglial activation was associated with the neuroprotective effect of Gal-1. As shown in Figure 3, Western blot analysis revealed significantly increased CCL2 expression in the Aβ-treated group compared to control rats (P<0.01); conversely, Ym1 expression was significantly decreased in the Aβ-treated rats (P<0.05).

Table 1. Cytokine levels in the hippocampus (pg/mg protein)

Cytokine	Control	Aβ	Gal-1L	Gal-1M	Gal-1H
IL-1β	36.85±2.59	47.35±2.57**	46.29±3.06	43.29±2.24##	42.32±1.84##
TNF-α	3.67±0.31	4.59±0.43**	4.29±0.41	4.02±0.16#	3.98±0.21##
IL-4	53.35±2.14	39.98±2.75**	42.18±2.24	43.73±2.98#	46.56±2.12##
IL-10	231.72±7.44	189.31±10.59**	199.53±8.48	212.13±9.74##	221.09±8.57##

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**P<0.01 vs the control group, #P<0.05 and ##P<0.01 vs the Aβ group.
Note: Cytokine levels were measured by ELISA.

Importantly, Gal-1 (5 and 10 μg) attenuated these changes induced by Aβ. Thus, Gal-1 influenced microglial polarization.

Effect of Gal-1 on hippocampal cytokines levels in AD rats

We used ELISAs to detect the concentrations of pro- and anti-inflammatory cytokines in the hippocampus. As shown in Table 1, compared to the control group, levels of IL-1β and TNF-α (proinflammatory factors) were significantly increased (P<0.01), while levels of IL-4 and IL-10 (anti-inflammatory factors) were decreased (P<0.01) in AD animals. These effects were attenuated by Gal-1 (5 and 10 μg) treatment.

Discussion

In this study, we used an AD model established in Sprague-Dawley rats treated with Aβ, a neurotoxin that activates microglia and induces AD-like pathology. We investigated the effects of Gal-1 on microglial polarization, and the results indicated that Gal-1 attenuated Aβ-induced M1 microglial polarization. Subsequently, neurotoxicity related to excessive M1 microglial activation—such as memory deficits, neuroinflammation, and neuronal injury—was improved after treatment with Gal-1.

Microglial activation is a hallmark that occurs in the central nervous system during the AD neurodegenerative process. In the brains of individuals with AD, Aβ triggers M1 microglial activation that exacerbates neuronal degeneration and death by releasing proinflammatory cytokines, whereas M2 microglial activation contributes to neuronal survival by generating neurotrophic factors, such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) and stimulating the clearance of amyloid plaques (Du et al., 2021; Yang et al., 2019; Wang et al., 2019). Therefore, strategies designed to reduce the overactivation of M1 microglia or promote microglial polarization toward the M2 phenotype would theoretically improve the outcome of neurodegenerative diseases.

The hippocampus is the most important structure in the pathophysiology of AD and is commonly examined in clinical and experimental studies of this disease (Palmer & Good, 2011). We first assessed histopathological changes in the hippocampus of Aβ-induced AD animals. Nissl staining revealed significant loss and shrinkage of neurons in AD rats compared to control rats, consistent with most previous reports (Zhao et al., 2019, Dai et al., 2018). However, neuronal damage mediated by Aβ was alleviated by Gal-1 (5 and 10 μg) treatment, indicating that Gal-1 exerted a repairing effect on damaged neurons. Therefore, we were not surprised to find that learning and memory deficits were attenuated in Gal-1-treated rats, indicating the potential ability of Gal-1 to improve AD.

Then, we assessed the effects of Gal-1 on the trends in Aβ-induced microglial M1 and M2 activation. The increase in CCL2 expression and the decrease in Ym1 expression induced by Aβ were significantly attenuated when the animals were treated with Gal-1. This result is similar to the findings reported by Suryawanshi et al., who observed that Gal-1 treatment shifted proinflammatory T cells toward an anti-inflammatory T cell type (Suryawanshi et al., 2013). The present changes in microglial activation status suggest that Gal-1 is involved in regulating microglial polarization in AD animals. Although the mechanism by which Gal-1 regulates microglial polarization was not explored in this study, previous experiments have found that the NF-κB pathway plays a role in microglial M1/M2 polarization (Wang et al., 2021; Chen et al., 2017). At the same time, Toegel et al. (2016) reported that Gal-1 possesses the ability to regulate NF-κB signaling. Therefore, we can speculate that NF-κB signaling is involved in the effect of Gal-1 on

microglial polarization, but further research is necessary to verify this hypothesis.

As stated above, neuroinflammation plays a key role in the pathological process of AD, and thus we analyzed the inflammatory changes in the hippocampus. In the current study, the levels of proinflammatory factors, such as IL-1β and TNF-α were substantially increased by Aβ infusion, while the secretion of anti-inflammatory factors, including IL-4 and IL-10, was suppressed. These results not only indicate that Aβ triggers neuroinflammation in AD animals but also support the hypothesis that Aβ induces an imbalanced M1/M2 activation pattern, since proinflammatory and anti-inflammatory factors are potential indicators of activated M1 and M2 microglial cells (Guo et al., 2022). Importantly, the secretion of proinflammatory cytokines was decreased, and the release of anti-inflammatory cytokines was increased by Gal-1 treatment. In this respect, our findings suggest that Gal-1 attenuates Aβ-initiated neuroinflammation by shifting microglial polarization from the M1 proinflammatory phenotype to the M2 anti-inflammatory phenotype.

Finally, not all Gal-1 concentrations were functional in the present study; the low dose of Gal-1 did not protect against pathological changes mediated by Aβ. Although high Gal-1 levels were more effective, the neuroprotective effects did not differ between medium and high Gal-1 doses. The effects of Gal-1 on neuroinflammation exhibited a dose-response "plateau" at the concentrations used in the present study; therefore, a higher dose may not be more effective for alleviating neuronal damage. We also noticed that high-dose Gal-1 treatment did not fully reverse neuronal injury mediated by Aβ. It is well known that, in addition to triggering neuroinflammation, Aβ is toxic to neurons in multiple ways. It can disrupt cellular calcium balance, cause pore formation resulting in the loss of membrane potential, and destroy the cytoskeleton (Reiss et al., 2018), while the effect of Gal-1 mainly focuses on immune regulation (Liu et al., 2012), which is probably the reason why Gal-1 cannot completely rescue neurons.

Conclusion

In summary, Gal-1 functions as a microglial modulator that suppresses microglial M1 polarization while favoring M2 polarization, thereby attenuating excessive inflammation and enhancing the neuroprotective effects of microglial cells. Although much more work is needed to further confirm the neuroprotective mechanisms of Gal-1 in AD—for example, the molecular mechanism by which Gal-1 regulates microglial polarization, which was not investigated in the present study—the protective effects of Gal-1 on AD-associated neuroinflammation may help provide the pharmacological basis for its future use in preventing the progression of neurodegenerative disorders.

Ethical Considerations

Compliance with ethical guidelines

The animal research protocols were reviewed and approved by the Animal Ethics Committee of Yunnan University, Kunming, China (Code: YNU-19-014). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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Authors' contributions

Methodology: Jiali Li and Liuyuan Deng; Investigation: Shufei Li, Yanmei Wang, Liuyuan Deng, and Jiali Li; Data acquisition: Shufei Li; Formal analysis: Shufei Li and Yanmei Wang; Writing the original draft: Yanmei Wang; Study design, funding acquisition, supervision, review, and editing: Yilong Dong; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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