Journal of Integrative Neuroscience

J. Integr. Neurosci. **2023**; 22(4): 92
https://doi.org/10.31083/j.jin2204092

Original Research

Probiotics Improve Cognitive Impairment by Decreasing Bacteria-Related Pattern Recognition Receptor-Mediated Inflammation in the Gut-Brain Axis of Mice

Xue-Qin Yang[1], Yang Zhao[1], Li Xue[2], Hui-Shan Wang[2], Jia Zeng[1], Jun-Rong Du[2,*], Zhe Xu[1,*]

[1]Department of Pharmacy, Guangyuan Central Hospital, Affiliated Hospital of North Sichuan Medical College, 628000 Guangyuan, Sichuan, China
[2]Department of Pharmacology, Key Laboratory of Drug-Targeting and Drug Delivery System of the Education Ministry, Sichuan Engineering Laboratory for Plant-Sourced Drug and Sichuan Research Center for Drug Precision Industrial Technology, West China School of Pharmacy, Sichuan University, 610041 Chengdu, Sichuan, China
*Correspondence: dujr_1@163.com (Jun-Rong Du); xuzhe_1122@163.com (Zhe Xu)
Academic Editors: Giovanna Traina and Gernot Riedel
Submitted: 25 October 2022 Revised: 8 December 2022 Accepted: 15 December 2022 Published: 7 July 2023

Abstract

Introduction: Some studies have found that probiotics can improve cognitive impairment in Alzheimer's disease, although the specific molecular mechanism by which this occurs has not been reported. Our previous research found that probiotics inhibited bacteria-related Toll-like receptor 4- and retinoic-acid-inducible gene-I-mediated nuclear factor-κB signaling pathways to improve cognitive impairment. However, it is unclear whether probiotics have similar effects on other pattern recognition receptors that respond to bacteria. **Methods**: Nine-month-old senescence-accelerated mouse prone 8 (SAMP8) mice received ProBiotic-4 (a mixture of *Lactobacillus acidophilus, Bifidobacterium bifidum, Lactobacillus casei*, and *Bifidobacterium lactis*) orally for 12 weeks. The effects on other bacteria-related pattern recognition receptors were then investigated. **Results**: ProBiotic-4-treated SAMP8 mice showed improvement in memory deficits, synaptic and cerebral neuronal injuries, and microglial activation. ProBiotic-4 also markedly increased the expression of intestinal tight junction proteins (i.e., claudin-1, occludin, and zonula occluden-1), decreased the expression of interleukin-1β at both the mRNA and protein levels, and reduced the expression of caspase-11, cleaved caspase-1, and α-kinase 1 (ALPK1) in the intestine and brain. **Conclusions**: These findings suggest that probiotics may have therapeutic potential for the treatment of inflammation in the gut-brain axis and for cognitive impairment. The mechanism of action of probiotics appears to be related to inhibition of the caspase-11/caspase-1 pathway and reduction of ALPK1 expression.

Keywords: gut-brain axis; cognitive impairment; inflammation; caspase 11/caspase 1; α-kinase 1

1. Introduction

The gut microbiota is comprised of more than 1000 different bacterial species [1] and plays an important role in gastrointestinal and brain functions [2]. It has been suggested the gut-brain axis is a bidirectional communication pathway because the brain can alter gut microbiota through the central nervous system, and the microbiota can regulate brain function through endocrine, immune, metabolic, and neuronal pathways [3,4]. Accumulating evidence suggests a clear relationship between the gut microbiota and cognitive function [5,6], thus raising the possibility that manipulation of gut microbiota may improve cognitive impairment.

Probiotics are mono- or mixed cultures of live microorganisms that are known to confer health benefits to the host when administered in adequate amounts [7]. Research into the effects of probiotics on cognitive function is increasing [8,9]. *Bifidobacterium breve* strain A1 suppressed the hippocampal expression of inflammation and prevented cognitive dysfunction in a mouse model of Alzheimer's disease (AD) [10]. Consumption of the probiotics *Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum*, and *Lactobacillus fermentum* for 12 weeks positively affected cognitive function in AD patients [9]. Furthermore, our previous research found that the mechanism by which probiotics improve cognitive impairment is associated with inhibition of the bacterial-related Toll-like receptor 4 (TLR4)- and retinoic-acid-inducible gene-I (RIG-I)-mediated nuclear factor κB (NF-κB) signaling pathways [11]. However, it is unclear whether probiotics have similar effects on other pattern recognition receptors that respond to bacteria.

Some bacterial products may cause cognitive deficits. For example, lipopolysaccharide (LPS) levels are significantly increased in the plasma of patients with dementia [12]. In addition to TLRs, the main receptors associated with LPS are caspases [13]. Murine caspase-11 (the ortholog of caspase-4 and caspase-5 in humans) is a caspase family member that responds to LPS, thereby mediating inflammatory responses and contributing to host defense mechanisms against Gram-negative bacteria [14,15]. Ac-

tivated caspase-11 has been shown to interact with caspase-1 to promote its activation and catalyze cleavage of the inactive interlukin-1β (IL-1β) precursor, thereby releasing mature IL-1β and contributing to neuroinflammation and neuronal loss [16–19]. Additionally, Zhou *et al*. [20] discovered a new pattern recognition receptor, α-kinase 1 (ALPK1), that can specifically recognize adenosine diphosphate (ADP)-heptose (i.e., an important component of LPS) and then activate the NF-κB pathway. In the current study, we investigated the effects of probiotics on the caspase-11/caspase-1 pathway and on ALPK1 expression, with the aim of elucidating the molecular mechanisms by which probiotics improve cognitive impairment.

We treated 9-month-old senescence-accelerated mouse prone-8 (SAMP8) mice, which develop neuronal loss and marked memory deficits [21,22], for 12 weeks with ProBiotic-4 (a mixture of *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Lactobacillus casei*, and *Bifidobacterium lactis*). ProBiotic-4 significantly reduced inflammation in the gut-brain axis and improved cognitive impairment in SAMP8 mice. The mechanism of action of ProBiotic-4 appears to be related to inhibition of the caspase-11/caspase-1 pathway and reduction of ALPK1 expression.

2. Materials and Methods

2.1 Materials

ProBiotic-4 was purchased from Swanson (198013, Fargo, ND, USA) and contains *Lactobacillus acidophilus* (12.5%), *Bifidobacterium bifidum* (12.5%), *Lactobacillus casei* (25%) and *Bifidobacterium lactis* (50%). The other reagents were of the highest quality available and were obtained from commercial sources.

2.2 Animals

Male 9-month-old senescence-accelerated mouse resistant 1 (SAMR1) and SAMP8 mice were purchased from the First Teaching Hospital of Tianjin University of Traditional Chinese Medicine (Tianjin, China). They were housed at 22 °C $\pm$ 2 °C in a 12 h/12 h light/dark cycle with free access to food and water. SAMP8 mice were divided randomly into two groups (12 per group) that received vehicle (water) or ProBiotic-4 (2×10^9 CFU/day in drinking water) for 12 weeks. Twelve SAMR1 mice were used as normal controls with similarly given vehicle. The animal studies were conducted in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China. All procedures were approved by the Animal Research Committee of West China School of Pharmacy.

2.3 Morris Water Maze Test

After 12 weeks of ProBiotic-4 treatment, the Morris water maze test was performed to assess spatial learning and memory ability in mice as previously described [23].

2.4 Tissue and Blood Collection

At the completion of the Morris water maze test, mouse plasma was collected for further analysis. The mice were perfused with cold normal saline via the ascending aorta, and cerebral and intestinal samples were collected for Western blotting and biochemical analyses.

2.5 Immunofluorescence Analysis

The expression of ionized calcium binding adapter molecule 1 (Iba-1) and synaptophysin (SYN) and neuron in per field of cerebral cortex or hippocampal CA1 was digitized as described previously [23,24]. Selected brain sections were incubated with the respective primary antibodies (Supporting Information **Supplementary Table 1**) at 4 °C overnight, followed by detection with a secondary antibody conjugated with fluorescein isothiocyanate (AR1177, Boster, Wuhan, Hubei, China). The area and the level of immunoreactivity per field of cerebral cortex or hippocampal CA1 was digitized (400$\times$ magnification) using Image Pro Plus 6.0 software (Media Cybernetics Corporation, Rockville, MD, USA).

2.6 Quantitative Real-Time Polymerase Chain Reaction

To determine IL-1β mRNA levels, total RNA first was isolated from mouse brain or intestinal tissues using TRIzol reagent (15596026, Thermo Fisher Scientific, Shanghai, China) and then processed for cDNA. Quantitative real-time polymerase chain reaction (qPCR) was performed according to our previously published methods [25]. The specific primer pairs are listed in Supporting Information **Supplementary Table 2**.

2.7 Enzyme-Linked Immunosorbent Assay

Plasma IL-1β levels were determined using mouse enzyme-linked immunosorbent assay (ELISA) kits (1210122, Dakewe Bioengineering, Shenzhen, China).

2.8 Western Blot Analysis

Protein samples were isolated from brain and intestinal tissue homogenates using RIPA buffer (P0013B, Beyotime, Shanghai, China) according to the manufacturer's instructions. The detailed procedure for Western blot analysis was described previously [25]. Primary antibodies against β-actin, claudin-1, occludin, zonula occluden-1 (ZO-1), caspase-11, caspase-1 and (-kinase 1 (ALPK1) were used to probe the respective target proteins. All antibodies and dilutions are listed in Supporting Information **Supplementary Table 1**.

2.9 Statistical Analysis

SPSS 19.0 software (IBM Corp., Chicago, IL, USA) was used for the statistical analysis. Data were expressed as the mean $\pm$ SD. One-way analysis of variance (ANOVA) with the Tukey *post hoc* test was used for statistical analysis. Compared with the vehicle-treated SAMP8 group, values of $p < 0.05$ were considered statistically significant.

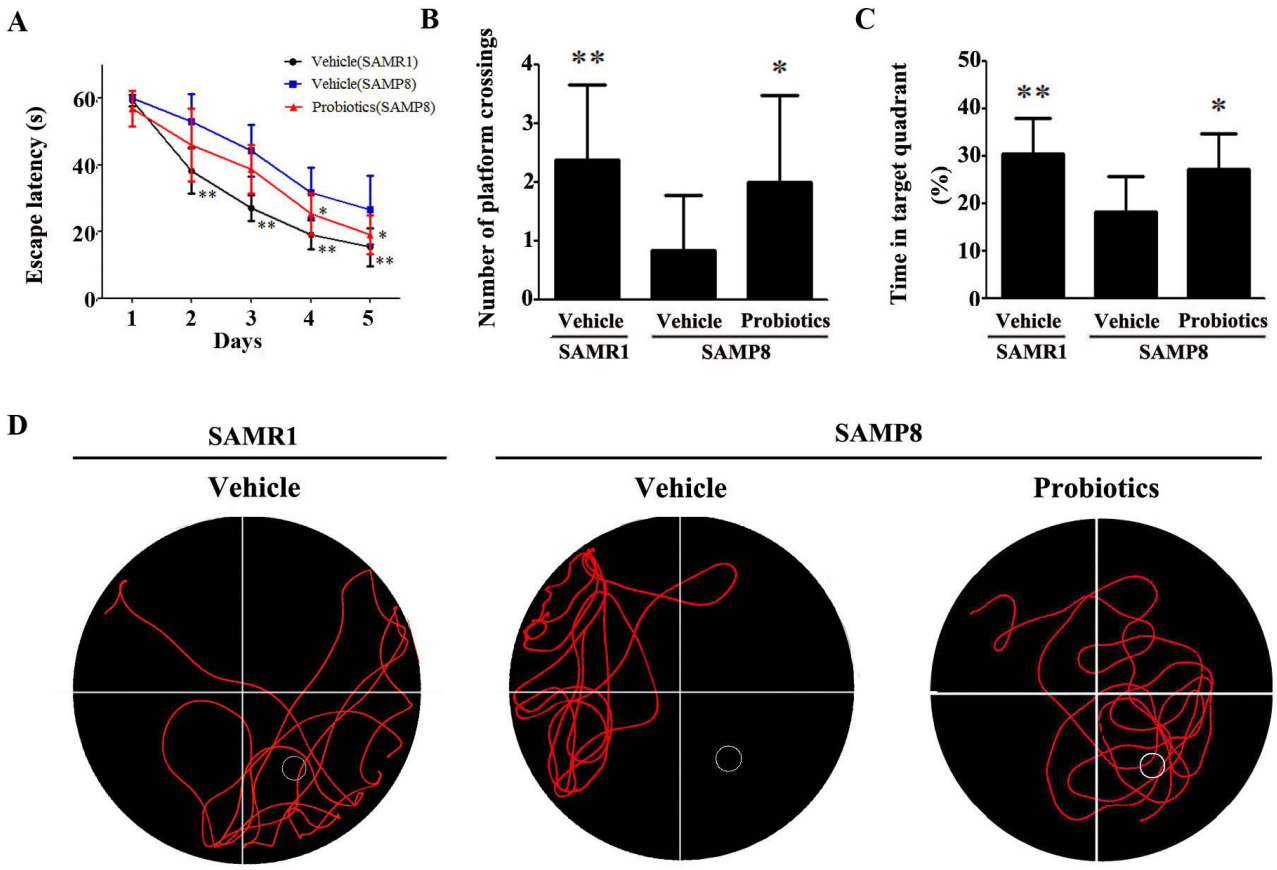

Fig. 1. ProBiotic-4 improved the performance of SAMP8 mice in the Morris water maze test. (A–C) Escape latency (A), number of platform crossings (B), and percent time in the target quadrant (C). (D) Characteristic swimming trails in the Morris water maze test. The data are expressed as mean ± SD (n = 10–12/group). *$p < 0.05$, **$p < 0.01$ *vs* vehicle-treated SAMP8 group (one-way ANOVA followed by Tukey *post hoc* test).

3. Results

3.1 ProBiotic-4 Improved the Performance of SAMP8 Mice in the Morris Water Maze Test

The Morris water maze test was used to examine spatial learning and memory in mice. In the hidden platform test (Fig. 1A), vehicle-treated SAMP8 mice exhibited a significantly longer escape latency compared with vehicle-treated SAMR1 controls ($p < 0.01$) during the overall training period. However, this was significantly shortened by probiotic treatment in the last 2 days of the training period ($p < 0.05$, *vs* vehicle-treated SAMP8). In the space exploration test (Fig. 1B–D), the number of platform crossings and time spent in the target quadrant were significantly decreased in the vehicle-treated SAMP8 group compared with the vehicle-treated SAMR1 group ($p < 0.01$). In SAMP8 mice, probiotics effectively increased these parameters compared with vehicle-treated mice, suggesting that probiotics improve cognitive impairment in these mice.

3.2 ProBiotic-4 Increased Synaptophysin Expression, Preserved Neuronal Survival, and Reduced Iba-1 Immunoreactivity in SAMP8 Mice

We examined the expression of SYN in the hippocampus and cortex. As shown in Fig. 2A, immunostaining revealed significantly lower SYN expression in the hippocampus and cortex of vehicle-treated SAMP8 mice compared with vehicle-treated SAMR1 mice ($p < 0.01$). Probiotics increased the level of expression in SAMP8 mice ($p < 0.05$). We also measured neuronal loss in the cortex. NeuN staining showed that the number of neurons in the cerebral cortex was significantly lower in vehicle-treated SAMP8 mice compared with vehicle-treated SAMR1 mice ($p < 0.01$). Probiotic treatment significantly increased the number of neurons in SAMP8 mice ($p < 0.01$; Fig. 2B). Additionally, we measured the activation of Iba-1 in the cerebral cortex. As shown in Fig. 2C, Iba-1 immunoreactivity was significantly higher in vehicle-treated SAMP8 mice compared with vehicle-treated SAMR1 mice ($p < 0.01$). Probiotic treatment significantly decrease Iba-1 immunoreactivity in SAMP8 mice ($p < 0.01$; Fig. 2C). These findings indicate that probiotic treatment significantly im-

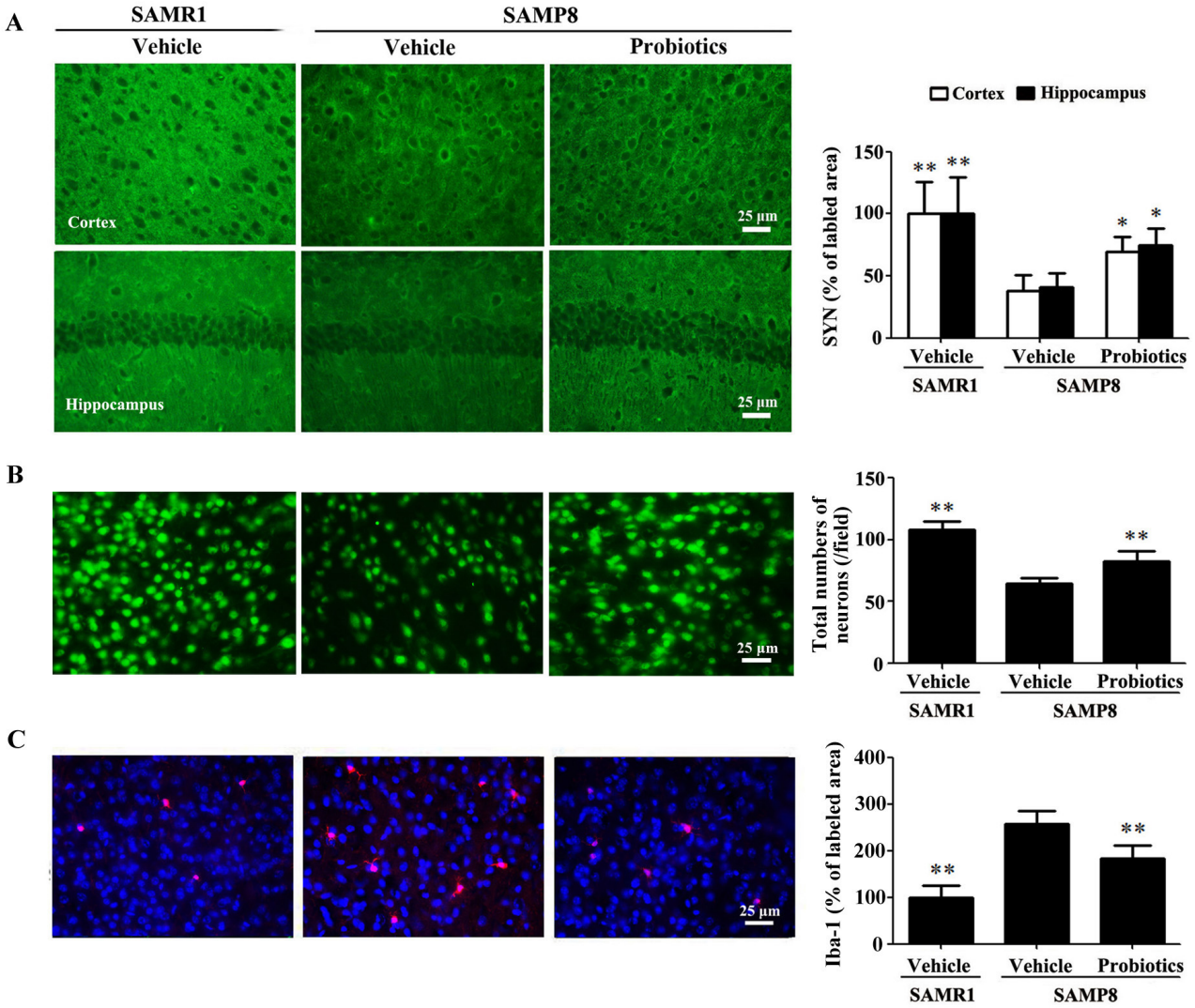

Fig. 2. ProBiotic-4 increased synaptophysin expression, preserved neuronal survival, and reduced Iba-1 immunoreactivity in SAMP8 mice. (A) Representative images of SYN immunofluorescence and quantitative analysis of the positive area of SYN expression in the cortex and hippocampal CA1 area of SAMR1 and SAMP8 mice. (B) Representative images of neuronal immunofluorescence and quantitative analysis of the number of NeuN-positive neurons in the cortex. (C) Representative images of Iba-1 immunofluorescence and quantitative analysis of the positive area of Iba-1 expression in the cortex. Scale bar = 25 μm. The data are expressed as mean ± SD (n = 5–6/group). *$p < 0.05$, **$p < 0.01$ *vs* vehicle-treated SAMP8 group (one-way ANOVA followed by Tukey *post hoc* test).

proves neuronal impairment and reduces neuroinflammation in SAMP8 mice.

3.3 ProBiotic-4 Increased the Expression of Intestinal Tight Junction Proteins in SAMP8 Mice

Tight junction proteins play a crucial role in maintaining the integrity of the intestinal barrier by effectively preventing harmful substances from entering the bloodstream [26]. We evaluated the effect of probiotics on the integrity of the intestinal barrier by examining the expression of intestinal tight junction proteins. Western blot analysis showed that expression of the claudin-1, occludin, and ZO-1 proteins was significantly lower in vehicle-treated SAMP8 mice compared with vehicle-treated SAMR1 mice ($p < 0.01$; Fig. 3). Probiotic treatment significantly increased the expression of intestinal tight junction proteins in SAMP8 mice ($p < 0.01$).

3.4 Probiotic-4 Inhibited the Caspase-11/Caspase-1 Pathway and Reduced the Expression of ALPK1 in SAMP8 Mice

Activation of the caspase-11/caspase-1 signaling pathway was detected in intestinal and brain tissues. As shown in Fig. 4A,B, the expression of caspase-11 and cleaved caspase-1 in the intestinal and brain tissues of vehicle-treated SAMP8 mice were significantly higher compared with vehicle-treated SAMR1 mice ($p < 0.01$). Probiotic treatment of SAMP8 mice significantly decreased the ex-

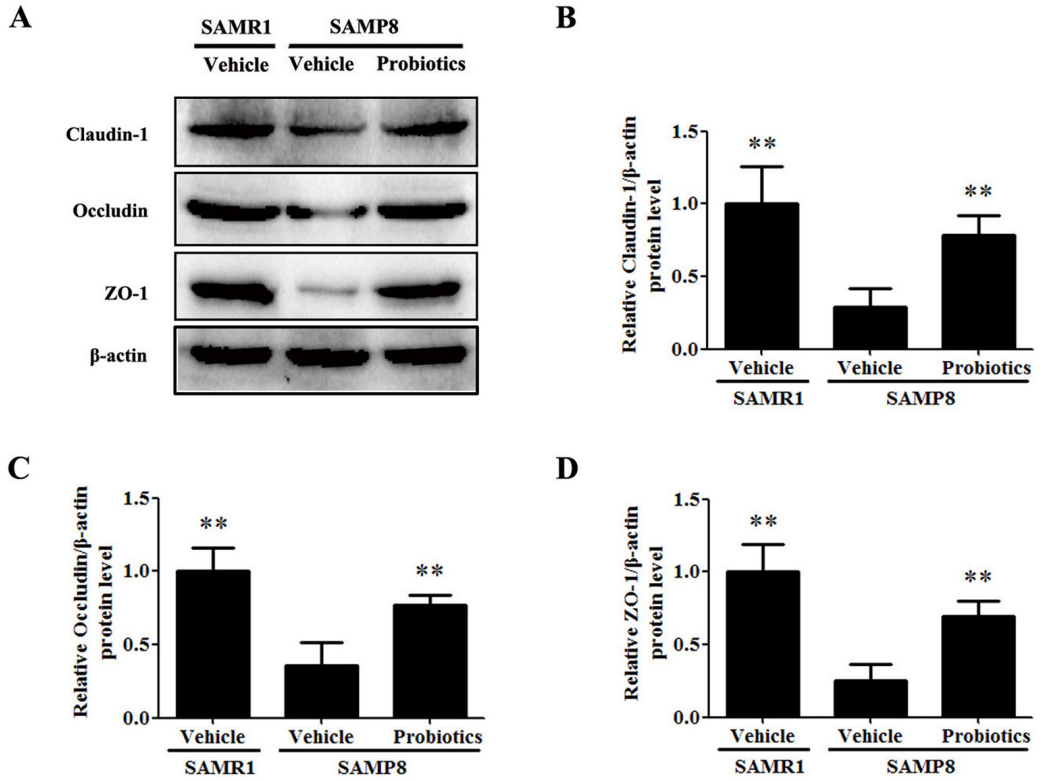

Fig. 3. ProBiotic-4 increased the expression of intestinal tight junction proteins in SAMP8 mice. (A) Protein expression of the intestinal tight junction markers claudin-1, occludin, and ZO-1 as detected by Western blot analysis in SAMR1 and SAMP8 mice. (B–D) Quantification of claudin-1, occludin, and ZO-1 protein expression in the intestine. The data are expressed as mean ± SD (n = 4/group). **$p < 0.01$ *vs* vehicle-treated SAMP8 group (one-way ANOVA followed by Tukey *post hoc* test).

pression of caspase-11 and cleaved caspase-1 in these tissues ($p < 0.05$). Activation of the caspase-11/caspase-1 signaling pathway produces mature IL-1β [27]. Therefore, we investigated the inflammatory cytokine IL-1β in the intestine, brain, and plasma. The mRNA expression of IL-1β decreased significantly in the intestine and brain of probiotic-treated SAMP8 mice ($p < 0.05$; Fig. 4C,D). The plasma level of IL-1β also decreased in probiotic-treated SAMP8 mice ($p < 0.05$; Fig. 4E). Moreover, ProBiotic-4 treatment significantly decreased the expression of ALPK1 protein in the intestine and brain ($p < 0.05$; Fig. 5). These findings indicate that probiotic treatment reduces inflammation in the gut-brain axis. The mechanism of action appears to involve the caspase-11/caspase-1 signaling pathway and expression of ALPK1.

4. Discussion

Although cognitive impairments are a common health problem in the elderly population, effective therapies for these conditions have yet to be discovered. Recent studies have drawn attention to the novel roles of probiotics in some gut-brain axis related conditions [28]. Some workers have reported that probiotics slowed the progression of cognitive decline in subjects with mild cognitive impairment [29], although the molecular mechanisms remain to be uncovered. Our previous research found that ProBiotic-4 could attenuate cognitive deficits in aged SAMP8 mice. The mechanism of action appeared to be related to the inhibition of TLR4- and RIG-I-mediated NF-κB signaling pathways that respond to bacteria. Therefore, in the present study we investigated further the effect of probiotics on other bacteria-related pattern recognition receptors. We found that oral ProBiotic-4 treatment for 12 weeks in SAMP8 mice significantly improved memory deficits, synaptic and cerebral neuronal injury, and microglial activation. Moreover, ProBiotic-4 treatment markedly increased the expression of intestinal tight junction proteins (i.e., claudin-1, occludin, and zonula occluden-1), decreased IL-1β expression at both the mRNA and protein levels, and reduced the expression of caspase-11, cleaved caspase-1, and ALPK1 in the intestine and brain. Overall, these observations indicate that the neuroprotective action of ProBiotic-4 against cognitive impairment is at least partly due to inhibition of the caspase-11/caspase-1 signaling pathway and to reduction of ALPK1 expression.

The present study used 9-month-old SAMP8 mice. These have previously been shown to exhibit cognitive deficits and neuronal injury compared with age-matched

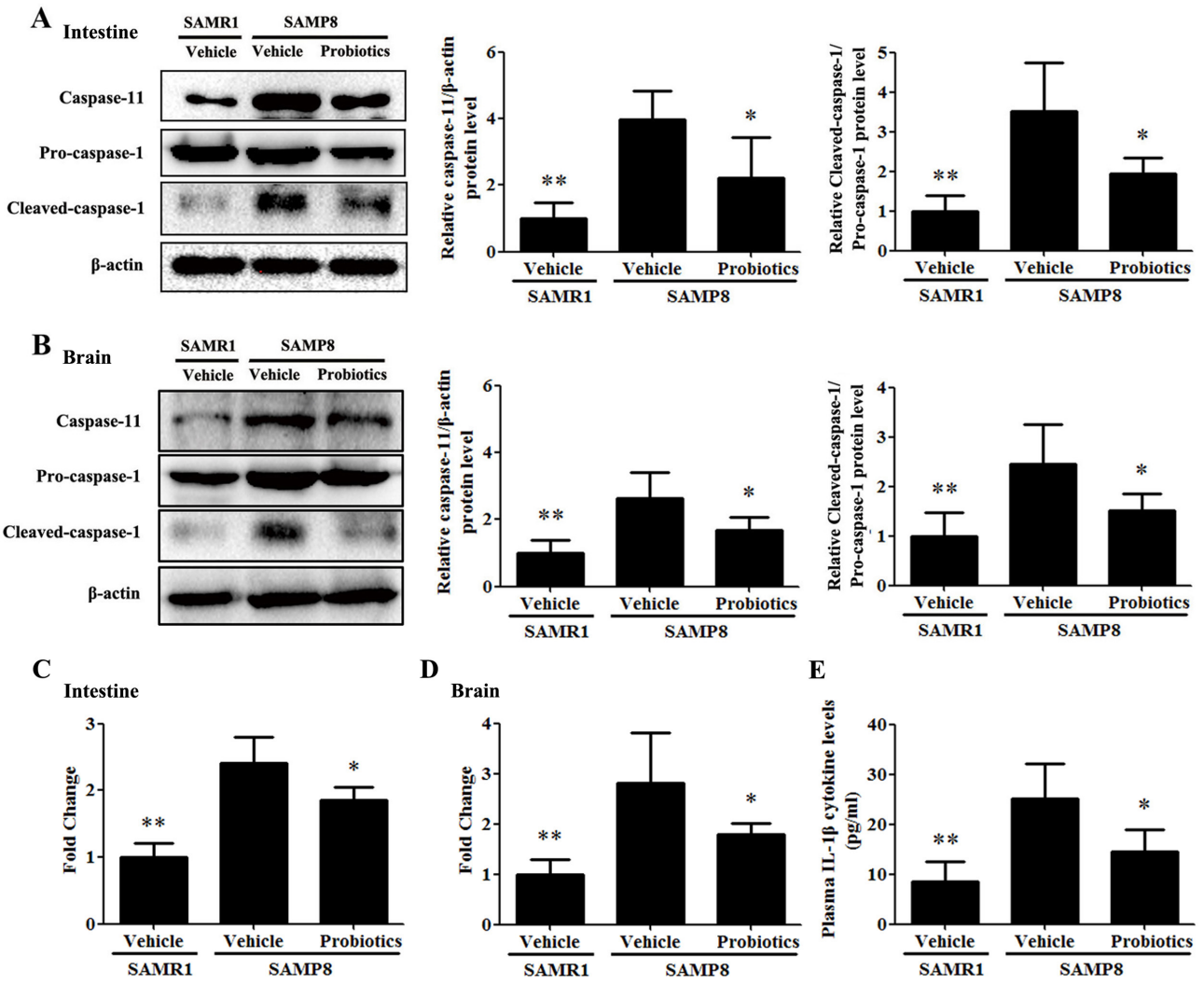

Fig. 4. Probiotic-4 inhibited activation of the caspase-11/caspase-1 pathway in SAMP8 mice. (A,B) Representative immunoblots and quantitative analysis of caspase-11, pro-caspase-1, and cleaved-caspase-1 expression in the intestine and brain of SAMR1 and SAMP8 mice. (C,D) Polymerase chain reaction analysis of IL-1β mRNA levels in the intestine and brain. (E) IL-1β levels in plasma were measured by ELISA. The data are expressed as mean ± SD (n = 4–5/group). *$p < 0.05$, **$p < 0.01$ *vs* vehicle-treated SAMP8 group (one-way ANOVA followed by Tukey *post hoc* test).

SAMR1 mice [21]. Similar to previous studies [30], vehicle-treated SAMP8 mice exhibited impairments in memory ability compared with age-matched SAMR1 mice. ProBiotic-4 treatment of SAMP8 mice significantly improved their spatial learning and memory ability, as determined by the Morris water maze test. ProBiotic-4 also improved neuropathological injuries in SAMP8 mice compared with vehicle-treated controls, including synaptic injury, neuronal loss and microglial activation, as well as reducing neuroinflammation in the brain. These findings suggest that ProBiotic-4 may improve cognitive impairment at least in part by inhibiting neuroinflammation.

Ever since the notion of a gut-brain axis was first proposed, the relationship between cognitive function and gut microbiota has become an active field of study. Dysbiosis of the gut microbiota can lead to an intestinal inflammatory response, intestinal barrier damage, and the intestinal leakage of proinflammatory cytokines, resulting in neuroinflammation [31]. Similar to the above studies, we observed increased IL-1β mRNA and protein levels and decreased expression of intestinal tight junction proteins in SAMP8 mice. Interestingly, ProBiotic-4 treatment of SAMP8 mice increased the expression of intestinal tight junction proteins and decreased IL-1β mRNA and protein levels. By limiting the decrease in expression of tight junction proteins, ameliorating intestinal inflammation and maintaining the integrity of intestinal barrier function, we found that ProBiotic-4 could attenuate cognitive impairment, consistent with previous studies [32,33].

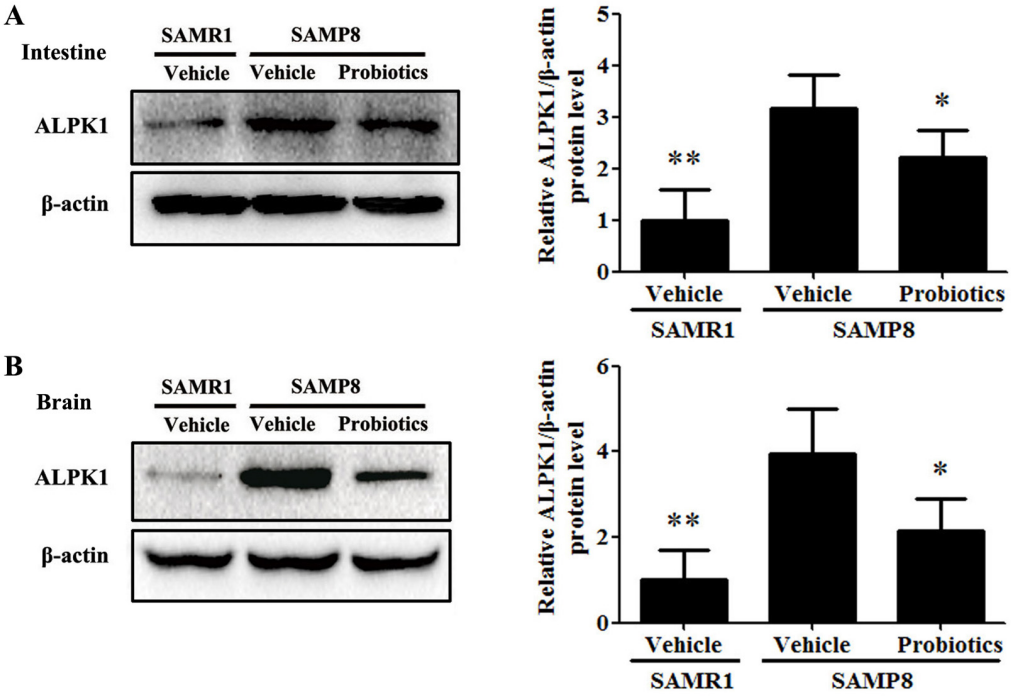

Fig. 5. Probiotic-4 decreased the expression of ALPK1 in SAMP8 mice. (A,B) Representative immunoblots and quantitative analysis of ALPK1 expression in the intestine and brain of SAMR1 and SAMP8 mice. The data are expressed as mean ± SD (n = 4/group). *p < 0.05, **p < 0.01 *vs* vehicle-treated SAMP8 group (one-way ANOVA followed by Tukey *post hoc* test).

When pathogenic bacteria invade the body, bacterial pathogen-associated molecular patterns (PAMPs) are recognized by special pattern recognition receptors and these activate the body's immune response [34]. LPS is a component of the Gram-negative bacterial cell wall and is also a PAMP that promotes activation of caspase-11 [35,36]. Together with caspase-1, caspase-11 is essential for the production of IL-1β and in most cells is only expressed upon induction with proinflammatory stimuli [36,37]. In a previous study, we found that gut-derived LPS reached the brain, resulting in a significantly increased LPS level in the brain of 12-month-old SAMP8 mice [11]. Therefore, to further explore the molecular mechanism by which ProBiotic-4 improves cognitive impairment, in the present study we investigated activation of the caspase-11/caspase-1 signaling pathway in SAMP8 mice. Compared with vehicle-treated SAMR1 mice, activation of the caspase-11/caspase-1 pathway was significantly upregulated in the intestine and brain of vehicle-treated SAMP8 mice. Moreover, ProBiotic-4 significantly reduced activation of the caspase-11/caspase-1 signaling pathway in SAMP8 mice. We also found that ProBiotic-4 reduced ALPK1 expression in the intestine and brain of SAMP8 mice. ALPK1 was first reported to specifically recognize ADP-heptose, a precursor metabolite in the synthesis of LPS, thereby activating the NF-κB pathway to mediate cytokine production [20]. The decreased expression of ALPK1 in probiotic-treated SAMP8 mice suggests that probiotics may reduce LPS production at the source, thereby attenuating the inflammatory response in the gut-brain axis. Collectively, the present results show that the effect of ProBiotic-4 on cognitive impairment is likely to be mediated by a reduction of inflammatory factors in the gut-brain axis. This suggests that probiotics may be a promising strategy for the prevention and treatment of gut-brain axis-related cognitive impairment.

5. Conclusions

In summary, the present study confirmed that the mechanism by which probiotics improve cognitive impairment involves inhibition of the caspase-11/caspase-1 signaling pathway, reduction of ALPK1 expression, and reduction of inflammation in the gut-brain axis. Our findings shed more light on the mechanism by which probiotics improve cognitive impairment, thereby providing an experimental basis for further research on their beneficial effects. Since a multi-strain formulation of probiotic was used in this study, it is unclear whether the mechanism of action is driven by one or more specific strains. Therefore, further comparisons between individual strains and multiple combinations need to be made to help understand the mechanism of action of probiotics on cognitive impairment.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Author Contributions

XQY—performed the research and drafted the manuscript; YZ and JZ—revised manuscript and made figures; LX—Validation and methodology; HSW—analysis of data; JRD—designed the work; ZX—revised manuscript, checked the statistical analysis and resulted analysis. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The procedures were approved by the Animal Research Committee of West China School of Pharmacy (approval number: SYXK(Chuan)2018-113).

Acknowledgment

Not applicable.

Funding

This work was supported by the National Natural Science Foundation of China (81473219 and 81973307) and partly by 111Project of the National Ministry of Education (B18035, China).

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.jin2204092.

References

[1] Sender R, Fuchs S, Milo R. Are We Really Vastly Outnumbered? Revisiting the Ratio of Bacterial to Host Cells in Humans. Cell. 2016; 164: 337–340.

[2] Gershon MD, Margolis KG. The gut, its microbiome, and the brain: connections and communications. The Journal of Clinical Investigation. 2021; 131: e143768.

[3] Gwak M, Chang S. Gut-Brain Connection: Microbiome, Gut Barrier, and Environmental Sensors. Immune Network. 2021; 21: e20.

[4] Dinan TG, Cryan JF. Gut instincts: microbiota as a key regulator of brain development, ageing and neurodegeneration. The Journal of Physiology. 2017; 595: 489–503.

[5] Kim C, Cha L, Sim M, Jung S, Chun WY, Baik HW, *et al*. Probiotic Supplementation Improves Cognitive Function and Mood with Changes in Gut Microbiota in Community-Dwelling Older Adults: A Randomized, Double-Blind, Placebo-Controlled, Multicenter Trial. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences. 2021; 76: 32–40.

[6] Junges VM, Closs VE, Nogueira GM, Gottlieb MGV. Crosstalk between Gut Microbiota and Central Nervous System: A Focus on Alzheimer's Disease. Current Alzheimer Research. 2018; 15: 1179–1190.

[7] Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, *et al*. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature Reviews. Gastroenterology & Hepatology. 2014; 11: 506–514.

[8] Steenbergen L, Sellaro R, van Hemert S, Bosch JA, Colzato LS. A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood. Brain, Behavior, and Immunity. 2015; 48: 258–264.

[9] Akbari E, Asemi Z, Daneshvar Kakhaki R, Bahmani F, Kouchaki E, Tamtaji OR, *et al*. Effect of Probiotic Supplementation on Cognitive Function and Metabolic Status in Alzheimer's Disease: A Randomized, Double-Blind and Controlled Trial. Frontiers in Aging Neuroscience. 2016; 8: 256.

[10] Kobayashi Y, Sugahara H, Shimada K, Mitsuyama E, Kuhara T, Yasuoka A, *et al*. Therapeutic potential of Bifidobacterium breve strain A1 for preventing cognitive impairment in Alzheimer's disease. Scientific Reports. 2017; 7: 13510.

[11] Yang X, Yu D, Xue L, Li H, Du J. Probiotics modulate the microbiota-gut-brain axis and improve memory deficits in aged SAMP8 mice. Acta Pharmaceutica Sinica. B. 2020; 10: 475–487.

[12] de Waal GM, Engelbrecht L, Davis T, de Villiers WJS, Kell DB, Pretorius E. Correlative Light-Electron Microscopy detects lipopolysaccharide and its association with fibrin fibres in Parkinson's Disease, Alzheimer's Disease and Type 2 Diabetes Mellitus. Scientific Reports. 2018; 8: 16798.

[13] Yang J, Zhao Y, Shao F. Non-canonical activation of inflammatory caspases by cytosolic LPS in innate immunity. Current Opinion in Immunology. 2015; 32: 78–83.

[14] Aachoui Y, Leaf IA, Hagar JA, Fontana MF, Campos CG, Zak DE, *et al*. Caspase-11 protects against bacteria that escape the vacuole. Science. 2013; 339: 975–978.

[15] Demon D, Vande Walle L, Lamkanfi M. Sensing the enemy within: how macrophages detect intracellular Gram-negative bacteria. Trends in Biochemical Sciences. 2014; 39: 574–576.

[16] Rothwell NJ, Luheshi GN. Interleukin 1 in the brain: biology, pathology and therapeutic target. Trends in Neurosciences. 2000; 23: 618–625.

[17] Kang SJ, Wang S, Hara H, Peterson EP, Namura S, Amin-Hanjani S, *et al*. Dual role of caspase-11 in mediating activation of caspase-1 and caspase-3 under pathological conditions. The Journal of Cell Biology. 2000; 149: 613–622.

[18] Manzoor Z, Koh YS. Caspase-11, the Main Executioner in Non-canonical Inflammasome. Journal of Bacteriology and Virology. 2012; 42: 169–171.

[19] Wang Y, Kasper LH. The role of microbiome in central nervous system disorders. Brain, Behavior, and Immunity. 2014; 38: 1–12.

[20] Zhou P, She Y, Dong N, Li P, He H, Borio A, *et al*. Alpha-kinase 1 is a cytosolic innate immune receptor for bacterial ADP-heptose. Nature. 2018; 561: 122–126.

[21] del Valle J, Bayod S, Camins A, Beas-Zárate C, Velázquez-Zamora DA, González-Burgos I, *et al*. Dendritic spine abnormalities in hippocampal CA1 pyramidal neurons underlying memory deficits in the SAMP8 mouse model of Alzheimer's disease. Journal of Alzheimer's Disease. 2012; 32: 233–240.

[22] Um MY, Choi WH, Ahn JY, Kim S, Kim MK, Ha TY. Sesaminol Glucosides Improve Cognitive Deficits and Oxidative Stress in SAMP8 Mice. Food Science and Biotechnology. 2009; 18: 1311–1315.

[23] Zeng C, Yang T, Zhou H, Zhao Y, Kuang X, Duan W, *et al*. Lentiviral vector-mediated overexpression of Klotho in the brain improves Alzheimer's disease-like pathology and cognitive deficits in mice. Neurobiology of Aging. 2019; 78: 18–28.

[24] Zhou H, Li H, Shi M, Mao X, Liu D, Chang Y, *et al*. Protective Effect of Klotho against Ischemic Brain Injury Is Associated

with Inhibition of RIG-I/NF-κB Signaling. Frontiers in Pharmacology. 2018; 8: 950.
[25] Kuang X, Chen Y, Wang L, Li Y, Liu K, Zhang M, *et al*. Klotho upregulation contributes to the neuroprotection of ligustilide in an Alzheimer's disease mouse model. Neurobiology of Aging. 2014; 35: 169–178.
[26] Lamprecht M, Frauwallner A. Exercise, intestinal barrier dysfunction and probiotic supplementation. Medicine and Sport Science. 2012; 59: 47–56.
[27] Py BF, Jin M, Desai BN, Penumaka A, Zhu H, Kober M, *et al*. Caspase-11 controls interleukin-1β release through degradation of TRPC1. Cell Reports. 2014; 6: 1122–1128.
[28] Ağagündüz D, Gençer Bingöl F, Çelik E, Cemali Ö, Özenir Ç, Özoğul F, *et al*. Recent developments in the probiotics as live biotherapeutic products (LBPs) as modulators of gut brain axis related neurological conditions. Journal of Translational Medicine. 2022; 20: 460.
[29] Sánchez-de-Lara-Sánchez S, Sánchez-Pérez AM. Probiotics Treatment Can Improve Cognition in Patients with Mild Cognitive Impairment: A Systematic Review. Journal of Alzheimer's Disease. 2022; 89: 1173–1191.
[30] Shi Y, Huang T, Chen L, Pan X, Zhang J, Zhu Y, *et al*. Ginsenoside Rg1 attenuates amyloid-beta content, regulates PKA/CREB activity, and improves cognitive performance in SAMP8 mice. Journal of Alzheimer's Disease. 2010; 19: 977–989.
[31] Yu W, Gao D, Wang Z, Jin W, Peng X, Zhao A, *et al*. Probiotics alleviate cognitive dysfunction associated with neuroinflammation in cardiac surgery. American Journal of Translational Research. 2019; 11: 7614–7626.
[32] Ghosh SS, He H, Wang J, Gehr TW, Ghosh S. Curcumin-mediated regulation of intestinal barrier function: The mechanism underlying its beneficial effects. Tissue Barriers. 2018; 6: e1425085.
[33] Wang Y, An Y, Ma W, Yu H, Lu Y, Zhang X, *et al*. 27-Hydroxycholesterol contributes to cognitive deficits in APP/PS1 transgenic mice through microbiota dysbiosis and intestinal barrier dysfunction. Journal of Neuroinflammation. 2020; 17: 199.
[34] Medzhitov R. Recognition of microorganisms and activation of the immune response. Nature. 2007; 449: 819–826.
[35] Moriyama K, Nishida O. Targeting Cytokines, Pathogen-Associated Molecular Patterns, and Damage-Associated Molecular Patterns in Sepsis via Blood Purification. International Journal of Molecular Sciences. 2021; 22: 8882.
[36] Schauvliege R, Vanrobaeys J, Schotte P, Beyaert R. Caspase-11 gene expression in response to lipopolysaccharide and interferon-gamma requires nuclear factor-kappa B and signal transducer and activator of transcription (STAT) 1. The Journal of Biological Chemistry. 2002; 277: 41624–41630.
[37] Kayagaki N, Warming S, Lamkanfi M, Vande Walle L, Louie S, Dong J, *et al*. Non-canonical inflammasome activation targets caspase-11. Nature. 2011; 479: 117–121.