ORIGINAL RESEARCH

Protective Effect of Rosmarinic Acid on Endotoxin-Induced Neuronal Damage Through Modulating GRP78/PERK/MANF Pathway

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Objective: Neuronal damage is criminal to cognitive dysfunction, closely related to endoplasmic reticulum stress (ERS). However, due to the pathogenesis of endotoxin-induced long-term cognitive dysfunction is not fully clarified, there is still a lack of effective treatment. This study was conducted to explore the protective effects and mechanism of rosmarinic acid (RA) against ERS in endotoxin-induced cognitive dysfunction in mice and neuronal injury in cells.

Methods: The efficacy of RA was evaluated using an endotoxin-induced cognitive dysfunction mice model and an in vitro neuronal injury model. Brain injury was assessed using behavioral tests and hematoxylin and eosin (HE) staining. Western blotting and Immunohistochemistry (IHC) were performed to determine NeuN, GRP78, PERK, ATF6, IRE1α, and MANF expression levels. Molecular docking was used to assess the associated mechanisms.

Results: Behavioral tests indicated that 20 and 40 mg/kg RA significantly improve endotoxin-induced cognitive dysfunction without dose differences. Histological analysis revealed no significant alterations in the number, morphology, and arrangement of neurons in the hippocampus and amygdala. However, 40 mg/kg RA treatment significantly decreased the hippocampal level of PERK protein and increased MANF in CA1 and DG in mice. Furthermore, our data showed that 120 μM RA pretreatment significantly inhibited LPS-conditioned culture-induced GRP78, PERK, and MANF upregulation in vitro. Finally, molecular docking studies suggested that RA could directly interact with GRP78, PERK, and IRE1, but not with MANF.

Conclusion: RA plays a protective role in improving cognitive function against endotoxemia-associated encephalopathy in mice via inhibiting the GRP78/PERK/MANF pathway.

Keywords: Rosmarinic acid, endotoxin, neuronal damage, GRP78/PERK/MANF pathway

Introduction

Persistent and uncontrolled systemic inflammatory response to bacteria, fungi, or viruses is the hallmark of sepsis, dramatically contributing to higher mortality, long-term cognitive impairment, and psychiatric disorders such as depression and anxiety.[1] In clinics, survivors of sepsis demonstrated significant deficits in learning and memory due to the reduction in left hippocampus and prefrontal cortex volume on MRI scans.[2] However, due to the pathogenesis of

systemic inflammation-induced long-term cognitive dysfunction is not fully clarified, there is still a lack of effective treatment.

Lipopolysaccharide (LPS) infusion is one of the main classes of sepsis animal models,[3] which can mimic septic long-term cognitive impairment and trigger sepsis-associated neuroinflammation in the different regions of the brain, including the hippocampus.[4] Currently, accumulating evidence has shown that neuronal damage is critical to the occurrence of cognitive dysfunction, which is closely related to endoplasmic reticulum stress (ERS) triggered by neuroinflammation.[5] In general, persistent or strong ERS causes massive aggregation of misfolded or unfolded proteins in the endoplasmic reticulum cavity, triggering ERS partner glucose-regulated protein 78 (GRP78) to dissociate from the three endoplasmic reticulum stress sensors, namely protein kinase R-like endoplasmic reticulum kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring enzyme 1α (IRE1α), and leads to calcium release, thereby initiating downstream cascades that result in inflammation.[6,7] A recent study has indicated that ERS inhibitor 4-phenyl butyrate (4-PBA) may control inflammation and notably ameliorate LPS-induced memory deficits in mice.[8]

Mesencephalic astrocyte-derived neurotrophic factor (MANF) is originally described as a neurotrophic factor, widely expressed in the developing and mature brain.[9] Recent studies have pointed out that the transcription of MANF is regulated by ER stress level owing to X-box binding protein 1 (XBP1) mainly binding to ER stress-responsive elements I (ERSEI) in the promoter region of MANF.[10] Additionally, increasing evidence demonstrated that endogenous MANF exerts its neuronal protection by regulating the UPR as a cofactor of GRP78 or directly binding with IRE1α.[11–14] In our previous study, we also found exogenous MANF supplementation inhibited ER stress in the lung tissue of mice via the GRP78/PERK/ATF4 pathway.[15] Therefore, MANF is a potential therapeutic target for many ER stress-related diseases.

Rosmarinic acid (RA) is a natural water-soluble polyphenol-containing hydroxy acid from the *comfrey, Labiatae, and Cucurbitaceae*, with anti-inflammatory, antioxidant, anti-sepsis, and other biological activities.[16] Our previous study found that RA could be a potential preventive medication for improving cognitive impairment and suppressing the endoplasmic reticulum stress mediated by the GRP78/IRE1α/JNK pathway.[17,18] However, in the central nervous system, whether RA regulates endoplasmic reticulum stress, especially the role of RA in modulating MANF, has not been reported. Therefore, we intend to explore whether MANF-mediated ERS is the potential target of RA in regulating endotoxin-induced neuronal injury.

In this study, different behavioral tests and histopathological analyses were employed to evaluate the protective effect of RA on endotoxin-induced neuronal injury in vivo. Besides, to demonstrate endotoxin-induced neuronal damage is mediated by microglial polarization, LPS-conditioned BV-2 media were applied in the SH-SY5Y cell culture. Next, we analyzed the MANF and ER stress-related protein levels in mice and SH-SY5Y cells to reveal the ideal therapeutic dose of RA. Finally, molecular docking and PPI networks were used to confirm the mechanism of RA against endotoxin-induced neuronal injury.

Materials and Methods

Ethics Statement

Animal ethics and experimental procedures were approved by Sichuan University and under the published guidelines of the China Council on Animal Care (No. K2022005).

Animals and Experimental Groups

Male (18–20 g) adult (6–8 weeks old) C57BL/6 mice (SPF grade) were obtained from Da-Shuo Biological Technology Co., Ltd (Chengdu, China) and held in standard environments in groups of 5 mice per cage and had ad libitum access to food and water. The mice were maintained on a 12 h (8 am-8 pm) light/dark cycle with consistent temperature (22 °C ± 1 °C) and humidity (55% ± 10%). All mice were randomly divided into the control group, LPS group, and rosmarinic acid low dose (RAL) and high dose (RAH) treatment group (n = 5).

Experimental Protocol

The mice in the RAL and RAH treatment group were administrated with RA (B20862, HPLC ≥ 98%, Yuanye, China) at 20 or 40 mg/kg once daily by intraperitoneal injection, while mice in the control group and LPS group were given the same volume of saline by intraperitoneal injection for 14 days (D1-D14). On day 7 (D7), LPS (*Escherichia coli* (O55: B5), Sigma Aldrich, USA) was administered at the dose of 10 mg/kg by intraperitoneal injection to induce the brain injury mouse model in the LPS and RA treatment groups. The control group received equal volumes of saline. From D21, the Open Field Test (OFT), Novel Object Recognition Test (NORT), as well as Elevated plus Maze Test (EPMT) were performed to assess their anxious state and memory abilities, respectively. The outline of the experimental protocol is shown in Figure 1.

Behavioral Tests

All the mice were habituated to the test room for 30 min before the test, and the observers were blinded to the group arrangement. Before each test, the arena was thoroughly sanitized using 75% alcohol.

OFT

The open field test was performed on day 21 to evaluate spontaneous locomotor activity and emotional change in a clean open-top chamber (40 × 40 × 40 cm) with a white floor. Each mouse was placed in the corner of the chamber and allowed to move freely for 5 min. The total distance traveled (cm) and the time spent in each square (s) were measured for 300 sec using the computerized video-tracking system (Taimeng Co, Chengdu, China) Each mouse was measured twice, with an interval of 4 hours between the two measurements.

NORT

On day 22, all mice were habituated to the NORT apparatus (40 × 40 ×40 cm) without objects for 5 min and then returned to their home cages 24 h before training. During the training session, mice were re-introduced to the arena to freely explore for 10 min in the presence of two familiar objects on the open field diagonal 8 cm away from the wall. The test session was carried either 1 h after the training session, and one of the familiar objects was replaced with a novel object. The mice were allowed to freely explore again for 5 min. The exploration time of mice for old and new objects was recorded. The recognition index (RI) was calculated using the following formula: RI = recognition time of new objects / (recognition time of new objects + recognition time of old objects) × 100%. Recognition is defined as touching and sniffing objects with forepaws or noses.

EPMT

On the 23rd day of the experiment, mice were subjected to the EPM test to evaluate potential signs of anxiety-related behavior. The EPM apparatus consisted of a white maze elevated 50 cm above the floor, with arms measuring 6 cm in width and 30 cm in length. For the test, each mouse was placed in the center of the maze and allowed to explore it for
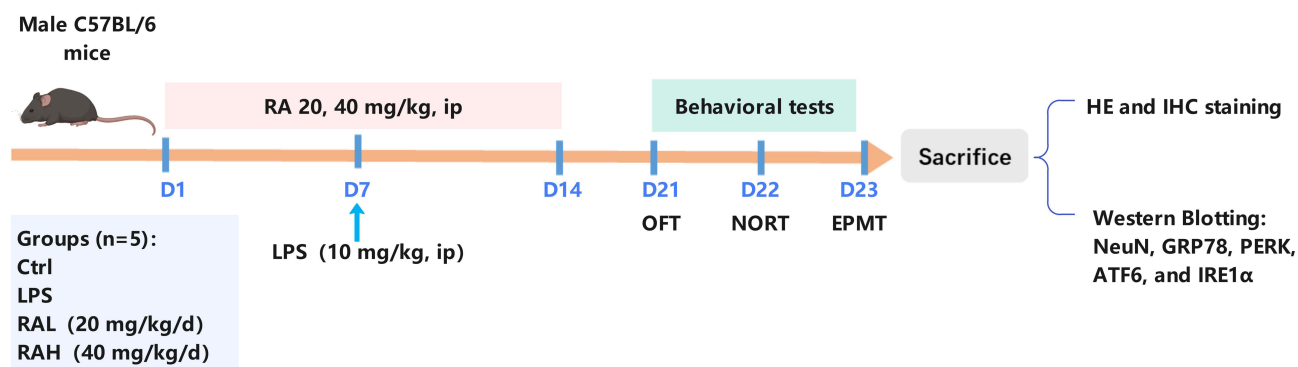

Figure 1 The outline of the experimental design.

5 min. The following parameters were analyzed: the total time spent in open arms and closed arms; and the number of times the animals entered the open arms.

Histopathological Assay

After being fixed in 4% paraformaldehyde at 4 °C for 48 h, the left brain was embedded in paraffin. These paraffin-embedded brain tissues were sliced into 4 μm-thick sections for hematoxylin and eosin (HE) staining. The light microscope (LEICA, DMi1, Wetzlar, Germany) was used to observe the histopathological changes.

Immunohistochemistry (IHC)

The above 4-μm sections were incubated with primary rabbit polyclonal antibody against MANF (1:400, ab67271, Abcam) at 4 °C overnight, followed by Goat anti-Rabbit-IgG Secondary Antibody (L3012, SAB) at room temperature for 50 min. After washing three times with PBS, DAB was used for IHC signal detection, then counterstaining with hematoxylin for 3 min. The images were captured by a light microscope (Leica DMil, Wetzlar, Germany) and the semi-quantitative analysis was performed blindly using Image J v1.52a software.

Cell Culture

SH-SY5Y were purchased from Pricella Biotechnology Co., Ltd. (Wuhan, China) and 5×10^5 cells/mL was seeded in 6-well plates for 24 h before different concentrations of RA (0, 40, 80, and 120 μM) pretreatments. BV2 cells were pretreated with LPS (0.2 μg/mL) for 24 h to collect the supernatant. After 4 hours of RA pretreatment, the BV2 cells supernatant was added to SH-SY5Y cell cultures, which were incubated for another 24 h.

Western Blot Analysis

Total proteins isolated from the right brain tissues or SH-SY5Y cells were lysed in RIPA Buffer (Beyotime Institute of Biotechnology, China). Then, proteins were separated by a 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene fluoride (PVDF) membrane (280 mA, 90 min). After being blocked with 5% no-fat milk at room temperature for 2 h, the membranes were probed with the diluted specific primary antibodies including NeuN (1:1000, Abcam), GRP78 (1:1000, HuaBio), PERK (1:1000, CST), ATF6 (1:1000, CST), IRE1α (1:1000, CST), and MANF (1:1000, Abcam) at 4 °C for 16 h, followed by incubation with horseradish peroxidase-conjugated secondary antibody (1:10000) at room temperature for 2 h. β-actin or GAPDH was used as the loading control. The protein was detected by chemiluminescence reagent (Millipore, USA), and the protein band density was quantified using Image J v1.52a software and was normalized to β-actin or GAPDH.

Molecular Docking and Protein-Protein Interaction (PPI) Network Analysis

Molecular docking was employed to investigate the potential interactions of RA with MANF, GRP78, PERK, and IRE1. The docking process was performed by CB-Dock2 (https://cadd.labshare.cn/cb-dock2/php/blinddock.php) to analyze the binding properties of the ligands for each protein.[19] The 3D structures of the 2w51 structural domain of MANF protein, the 6hab structural domain of GRP78 protein, the 4yzs structural domain of PERK protein, and the 6shc structural domain of IRE1 protein were downloaded from the PDB database (RCSB PDB: Homepage) and exported in PDB format. To obtain potential interacting proteins related to MANF, GRP78, PERK, and IRE1, the Retrieval of Interacting Genes/Proteins (STRING) version 11.0 database (https://string-db.org/) and GeneMANIA (http://genemania.org/) were used.

Statistical Analysis

Statistical analyses were performed with the GraphPad Prism 8.0 software. Continuous and normally distributed variables were expressed as the mean ± SD and analyzed by Student's t-test or one-way analysis of variance (ANOVA) with Bonferroni post hoc test. Non-normal distribution data using Wilcoxon signed rank test (OFT and IHC data) and Kruskal Wallis H rank combination test (others). $P < 0.05$ was considered statistically significant.

Results

Effect of RA on Endotoxin-Induced Brain Injury in Mice

Preventive and Therapeutic Effects of RA on Endotoxin-Induced Cognitive Dysfunction in Mice

In the OFT: The distribution of motion trajectories in the control mice was relatively uniform throughout the open field (Figure 2A), and the difference in the residence time of mice in the central region, edge region, and corner region was not obvious (P>0.05, Figure 2B). The model group showed an uneven distribution of motion trajectories compared to the control group (Figure 2A). There was a significant decrease in total motion distance and residence time in central and side areas, and an increase in resting time and residence time in the corner areas (P<0.05, Figure 2B). Nevertheless, the motion trajectory distribution was more uniform in both doses of RA treatment compared to the model group (Figure 2A), accompanied by the reduced total resting time and increased residence time in the central area (P<0.05, Figure 2B). Although there were no statistically significant differences in the residence time within the corner area, there was a clear decreasing trend.

In the EPMT: As shown in Figure 2A and C, the time spent in the closed and open arms of the elevated plus maze was similar in the control group. The model group hardly entered the open-arm in the EPM and showed a significant increase in resting time and a decrease in open-arm residence time percentage (P<0.01, Figure 2C). RA treatment led to a significant increase in the number of entries and time spent in the open arms of the EPM (P<0.01, Figure 2C). Moreover, there was no significant difference between the two doses of RA treatment (P>0.05, Figure 2C).

In the NORT: The discrimination index of mice in the control group was higher than the random level (50%), which was significantly higher than the model group (P<0.01, Figure 2D). After RA treatment, the discrimination index significantly increased compared to the model group (P<0.05, Figure 2D) with no dose differences.

Effect of RA on Brain Histopathology

To further confirm the effect of RA on endotoxin-induced brain injury, we analyzed the number and structure of neurons in the hippocampus (HIP) and amygdala (AMY) by HE staining. As shown in Figure 3A, no significant alters were observed in the number, morphology, and arrangement of neurons in the hippocampus and amygdala in any of the groups. Also, there was no significant difference in the level of NeuN protein in the hippocampus of mice between each group (P>0.05, Figure 3B). All of these suggest that the hippocampus and amygdala do not experience significant
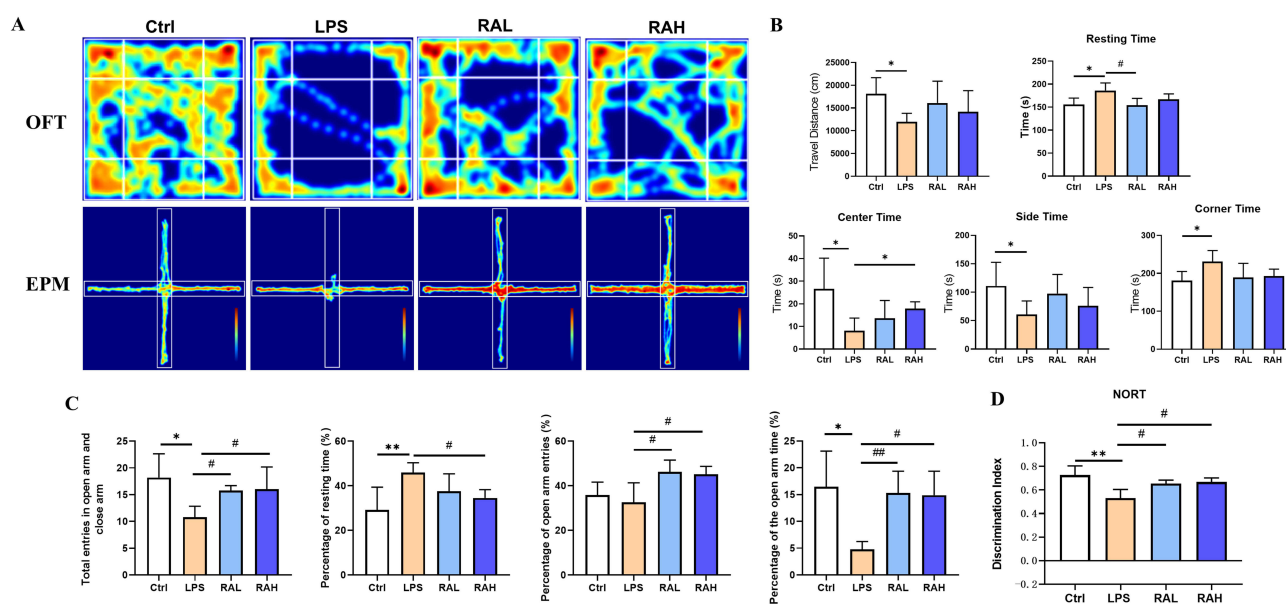

Figure 2 Effect of RA on endotoxin-induced cognitive dysfunction in mice. (**A**) Represent moving tracks in the open-field test (OFT); (**B**) Travel distance (upper Left panel), resting time (upper right panel), center time (lower left panel), side time (lower middle panel), and corner time (lower right panel) in the OFT; (**C**) Different index in the EMPT; (**D**) The discrimination index and the total exploration time in the NORT. Data are means ± SD. *p < 0.05, **p < 0.01 compared with the Ctrl group; #p < 0.05, ##p < 0.01 compared with the LPS group (n=5).
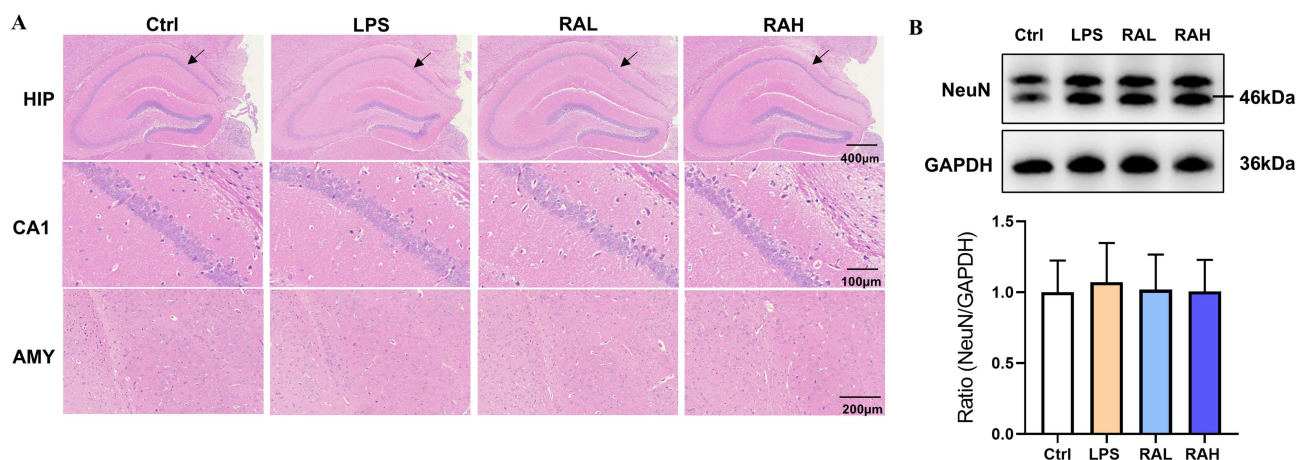
Figure 3 Effect of RA on endotoxin-induced brain injury in mice. (**A**) Represent HE stains in the hippocampus (Hip), CA1 region, and amygdala (AMY) of mice; the black arrows indicate the regions that have been magnified in the second row for a detailed visualization; (**B**) Representative Western blots (upper panel) and quantitative analysis (upper panel) of NeuN protein in the hippocampus. GAPDH was used as an internal standard. Data are means ± SD. *p < 0.05 compared with the Ctrl group; #p < 0.05 compared with the LPS group (n=5).

neuronal death following endotoxin-induced brain injury after 14 days. The cognitive decline observed in mice may be due to impaired neuronal function.

RA Against Endotoxin-Induced Brain Injury in Mice via Inhibiting ERS

RA Ameliorated Endotoxin-Induced Hippocampal ERS

To evaluate the effect of RA on endotoxin-induced ERS, the protein levels of ERS-related markers, such as glucose-regulated protein 78 (GRP78), protein kinase RNA-like ER kinase (PERK), inositol-requiring enzyme 1α (IRE1α), and activating transcription factor 6 (ATF6) in the hippocampus were measured by Western blotting analysis. Our data indicated that endotoxin significantly increased the expression of GRP78 and PERK in the hippocampus compared to the control group (p<0.01, Figure 4). However, only high-dose RA treatment significantly decreased the hippocampal level
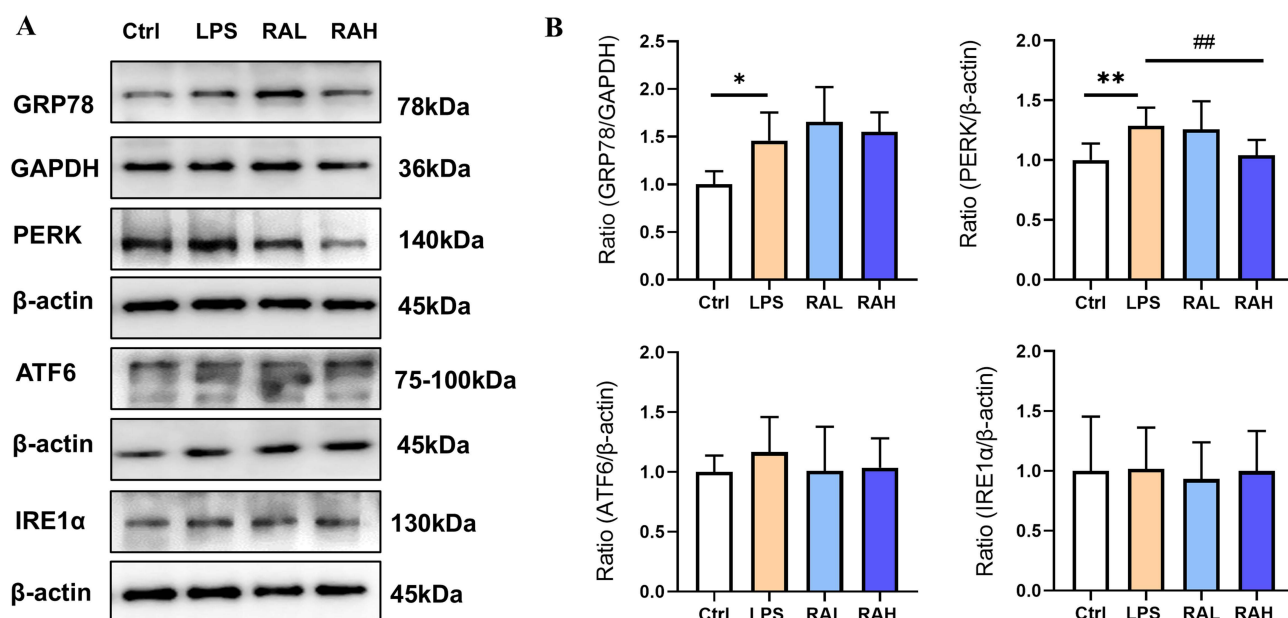

Figure 4 Effect of RA on ER stress-associated proteins in the hippocampus of mice. (**A**) Representative Western blots and (**B**) quantitative analysis of GRP78, PERK, ATF6, and IRE1a protein in the hippocampus. β-actin or GAPDH was used as an internal standard. Data are means ± SD. *p < 0.05, **p < 0.01 compared with the Ctrl group; ##p < 0.01 compared with the LPS group (n=5).

of PERK protein (P<0.01). The low-dose group had no significant difference in their PERK level (P>0.05, Figure 4). Regarding GRP78, RA treatment did not significantly reverse its expression compared to the model group (P>0.05, Figure 4). On the other hand, the hippocampal protein levels of ATF6 and IRE1α in each group showed no significant difference (P>0.05). These findings suggest that the endotoxin-induced ERS in the hippocampus is mainly associated with the PERK pathway.

Effect of RA on MANF Expression in Hippocampus and Amygdala

In the present study, we found the hippocampal protein levels of MANF in each group showed no significant difference (P>0.05, Figure 5). However, compared to the control group, the expression of MANF in the amygdala of the model group was significantly increased (P<0.05, Figure 5), although this was not reversed by RA treatment (P>0.05, Figure 5). Considering that the hippocampus includes multiple regions that regulate different learning and memory functions, we counted the expression of MANF protein in the CA1, CA2, CA3, and DG regions, respectively. According to our findings, the expression of MANF in DG was significantly increased by endotoxin (P<0.05, Figure 6), whereas RAH
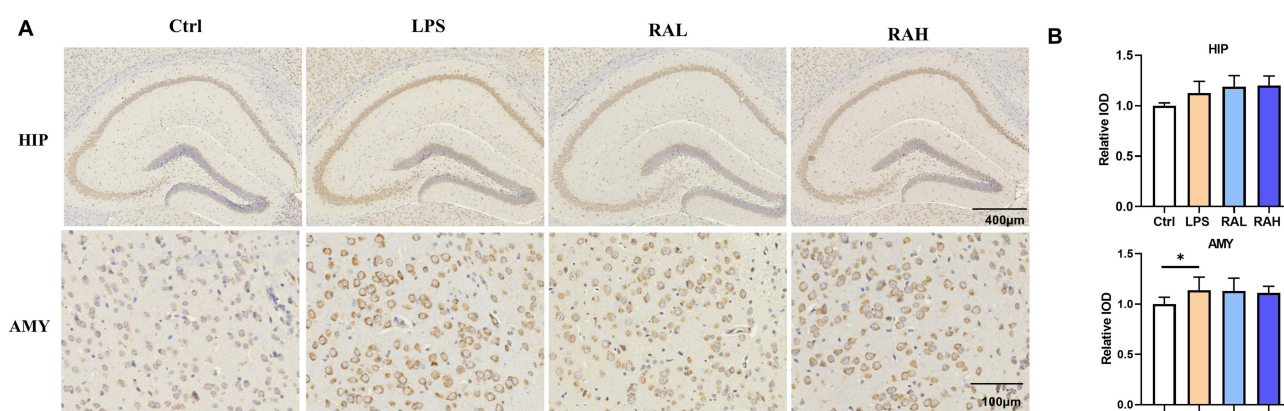

Figure 5 Effect of RA on MANF expression in the hippocampus and amygdala of mice. (**A**) Representative IHC images of MANF in the hippocampus (Hip) and amygdala (AMY) of mice; (**B**) Semi-quantitative analysis. Data are means ± SD. *p < 0.05 compared with the Ctrl group (n=5).
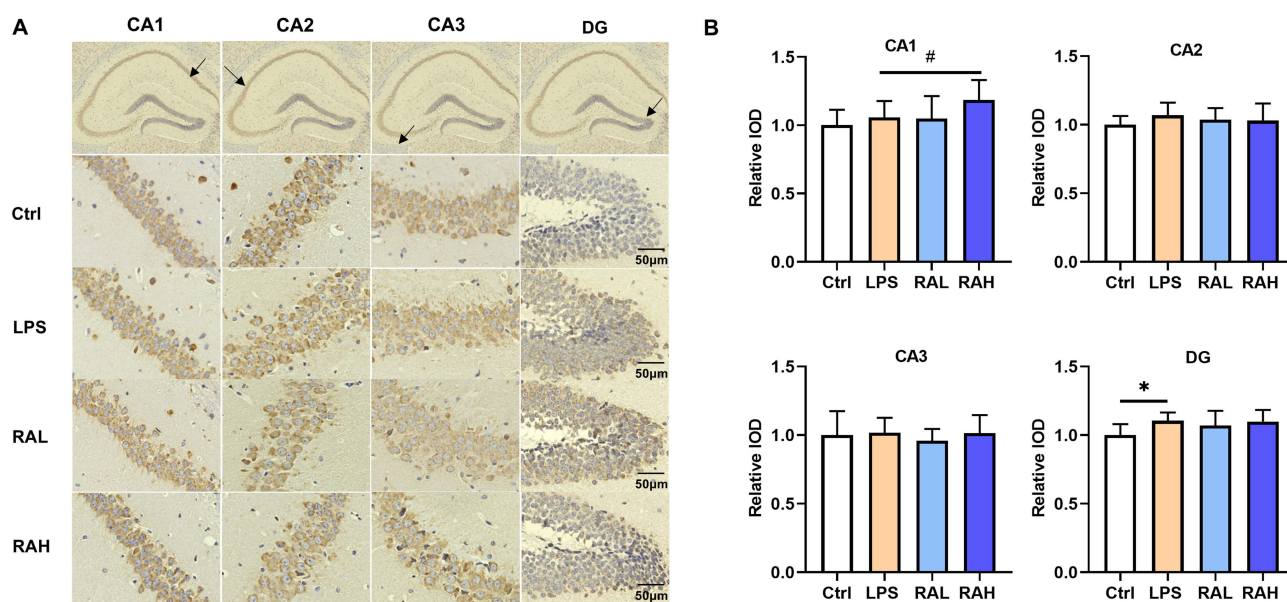

Figure 6 Effect of RA on MANF expression in different regions of the hippocampus. (**A**) Representative IHC images of MANF in the CA1, CA2, CA3, and DG of the hippocampus; the black arrows indicate the regions that have been magnified in the subsequent rows for a detailed visualization; (**B**) Semi-quantitative analysis. Data are means ± SD. *p < 0.05 compared with the Ctrl group; #p < 0.05 compared with the LPS group (n=5).

significantly increased MANF in CA1 and DG when compared to the control group (P<0.05, Figure 6). In addition, protein levels of MANF in CA2 and CA3 did not show any notable difference in all groups (P>0.05, Figure 6). Regional differences in MANF expression suggested that RA treatment enhanced various forms of memory, especially long-term learning, and memory of sound, light, taste, and other events via up-regulating MANF.[20]

RA Inhibits Endotoxin-Induced Neuronal ERS via GRP78/PERK/MANF Pathway

To further determine the effect of RA on neuronal MANF and ERS-related proteins, neuronal SH-SY5Y was cultured in vitro. First, we used the CCK8 method to detect the activity of SH-SY5Y cells in the case of different concentrations of RA treatment for 24 h. As shown in Figure 7A, with an increase in RA administration concentration from 40 μM to 160 μM, the activity of SH-SY5Y cells remained unchanged. Therefore, RA concentrations of 40 μM, 80 μM, and 120 μM were used in the subsequent experiments. As expected, LPS-conditioned culture dramatically increased in GRP78, PERK, and MANF expressions in neuronal SH-SY5Y cells (P<0.001, Figure 7B and C). Moreover, 120 μM RA pretreatment significantly inhibited LPS-conditioned culture-induced GRP78 and PERK upregulation (P<0.001, Figure 7B and C). MANF level also showed a significant downward trend (P = 0.051, Figure 7B and C). However, the expressions of GRP78 and PERK were not altered by 40 and 80 μM RA pretreatment (P>0.05), suggesting the high-concentration RA pretreatment can inhibit ERS and the expression of MANF in SH-SY5Y cells in the LPS co-culture system.
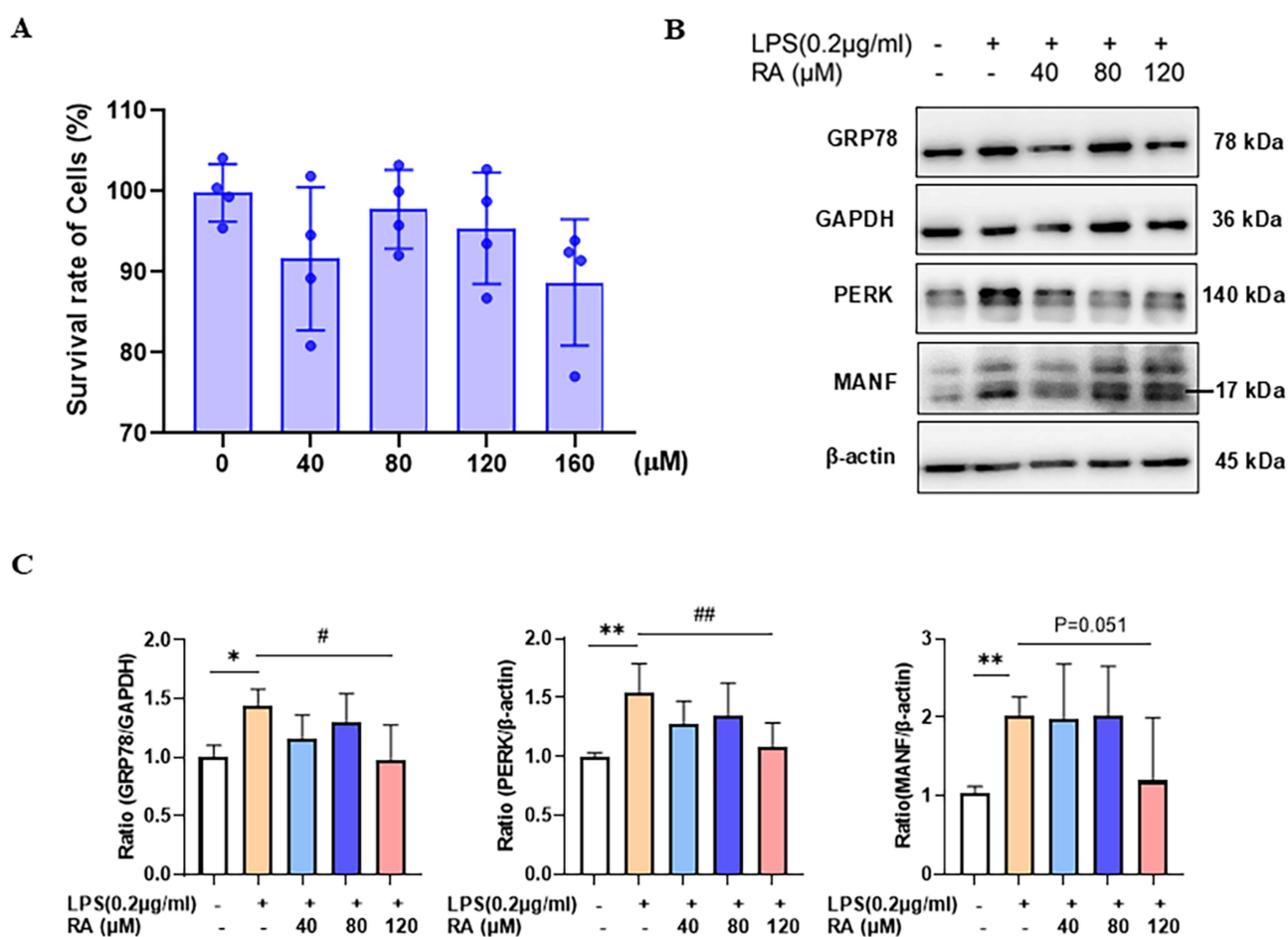

Figure 7 Effect of RA on endotoxin-induced neuronal ERS in vitro. (**A**) Survival rate in SH-SY5Y cells; (**B**) Representative Western blots and (**C**) quantitative analysis of GRP78, PERK, and MANF protein in SH-SY5Y cells. β-actin or GAPDH was used as an internal standard. Data are means ± SD. *p < 0.05, **p < 0.01 compared with the Ctrl group; #p < 0.05, ##p < 0.01 compared with the LPS group (n=5).

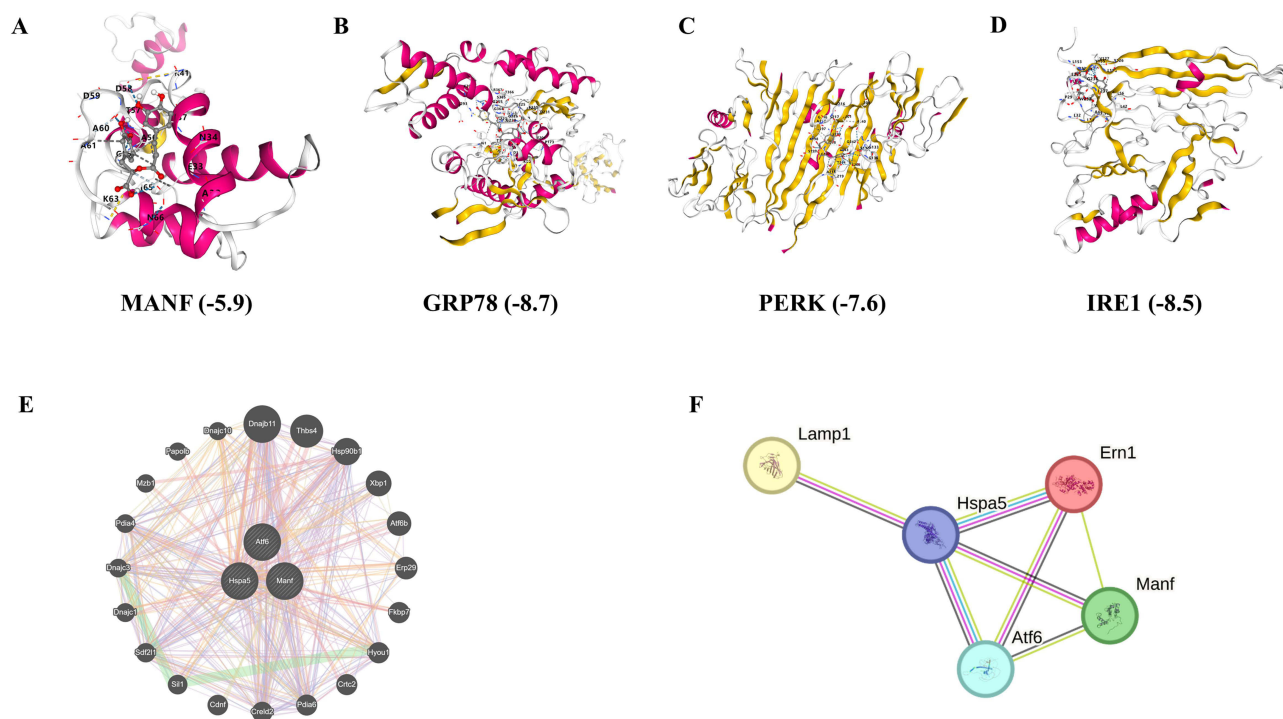**Figure 8** Mechanism of RA on endotoxin-induced neuronal ERS. Molecular docking (CB-Dock2) among RA with GRP78 (**A**), PERK (**B**), IRE1 (**C**), and MANF (**D**); GeneMANIA (**E**) and STRING (**F**) PPI network analysis.

Finally, molecular docking studies were conducted to confirm if the RA interacts directly with MANF or ERS-related proteins. Interestingly, RA had a higher Vina score to MANF (−5.9, Figure 8A) than GRP78 (−8.7, Figure 8B), PERK (−7.6, Figure 8C), and IRE1 (−8.5, Figure 8D), suggesting that RA was easy to interact with GRP78, PERK, and IRE1 directly, but not with MANF. The STRING database and GeneMANIA also revealed a protein interaction network between MANF and biomarkers of endoplasmic reticulum stress as shown in Figure 8E and F.

Discussion

Our previous study indicated pretreatment of RA at the dose of 20 mg/kg by intraperitoneal injection could prevent cognitive impairment in CLP-induced sepsis mice models,[17] which suggested that RA is a potential novel preventive drug for improving cognitive impairment in sepsis survivors since it demonstrated a significant amelioration effect on glycolytic metabolism abnormality via regulating microglial polarization. However, as executors of various neural functions, damage to neurons is essential in the development of cognitive impairment.[5] In this study, we attempted to reveal the effective dose of RA on endotoxin-induced neuronal damage from the perspective of ER stress. We found that 20 or 40 mg/kg RA once daily by intraperitoneal injection for 14 days improves the anxiety-like behavior of endotoxemia-associated encephalopathy mice in OFT and EPMT, whereas increases the discrimination index of mice for novel objects. Moreover, 20 or 40 mg/kg RA exhibits similar effects in behavioral evaluation.

The hippocampus is one of the areas most affected by neuroinflammation and oxidative stress in endotoxemia-associated encephalopathy.[21] Also, as the key hub for processing stress and fear in the brain, the amygdala is the key biological basis for the occurrence of anxiety.[22] Therefore, the basolateral amygdala (BLA) and hippocampus were verified to play crucial roles in regulating anxiety-like behaviors.[23] In the present study, our data indicated that LPS 10 mg/kg single exposure did not alter the structure, arrangement, and number of neurons in the amygdala and hippocampus 16 days later, which is not consistent with the results of behavioral tests. We thought intraperitoneal injection of 10 mg/kg LPS was not enough to cause significant pathological changes in hippocampal neurons on the 16th day after injection. The endotoxin-induced anxiety-like mood and abnormal recognition and memory ability in mice may be mainly related to the early neuronal functional changes in the hippocampus and amygdala. As research has found,

before the loss of hippocampal neurons in Alzheimer's disease, the connectivity of the hippocampal structure that contributes to the vulnerability of these circuits has been compromised for a long time.[24]

Endotoxin-induced excessive activation of ER stress is crucial in mediating neuronal death.[25] GRP78 was associated with anti-inflammatory responses, which was suggested as a potential therapy for endotoxin-induced organ injury, including lung injury[15,18] and neuron injury.[26] Also, LPS-enhanced UPR led to decreased cell viability in a time-dependent manner and dose-dependent manner.[26] In the current research, endotoxin significantly increased the hippocampal GRP78 and PERK levels, but not ATF6 and IRE1α. It is interesting that only high-dose RA treatment significantly decreased the hippocampal PERK protein level, implying that the endotoxin-induced ERS in the hippocampus is mainly associated with the PERK pathway, which is also the potential therapeutic mechanism of RA. We thought the reason is the PERK pathway is the preferred activation pathway induced by ERS compared to ATF6 and IRE1α.[27] However, 40 mg/kg RA did not inhibit endotoxin-induced GPR78 upregulation in the hippocampus, which is not consistent with our finding in lung tissues.[15] This difference might be owing to the tissue or cell type specificity or the dose dependence of RA use because high concentrations of RA can significantly downregulate the levels of GRP78 and PERK proteins in cultured SH-SY5Y cells in vitro.

MANF is an ER chaperone protein, mainly interacting with GRP78. MANF has multiple effects on ER stress, including interaction directly with one or more UPR sensors,[14] and regulation of PERK and IRE1 pathways by directly binding to specific endoplasmic reticulum luminal domains without interaction with GRP78.[12] Our previous study demonstrated that MANF as a nucleotide exchange inhibitor to GRP78 suppresses the GRP78/PERK/ATF4 axis in sepsis-associated lung injury.[15] Inconsistent with our expectations, we found that MANF in the amygdala and DG of the model group was significantly increased, although there is no statistical difference in the other area of the hippocampus. A similar result was shown in neuronal SH-SY5Y cells that LPS-conditioned culture dramatically increased MANF expression. Interestingly, a high dose of RA significantly increased MANF in CA1 and DG region in mice, whereas decreased MANF expression in vitro. Combining molecular docking results RA was able to interact with GRP78, PERK, and IRE1, but not with MANF, we thought RA plays its neuroprotective role by regulating GRP78/PERK/MANF pathway. Among that, the MANF level is dependent on GRP78. On the contrary, PERK expression is directly regulated by RA, which is independent of the GRP78 level.

Conclusions

We demonstrated that endotoxin-induced ERS in the hippocampus is mainly associated with the PERK pathway, which contributes to endotoxemia-associated encephalopathy in mice. RA against endotoxemia-associated encephalopathy in mice via inhibiting the GRP78/PERK/MANF pathway, thus playing a protective role in improving cognitive function. However, this study also has some limitations. First, it did not provide evidence for how RA targeted GRP78 and PERK. Second, brain area and cell type specificity of GRP78 and MANF must be further investigated. Thus, further studies will help establish the precise mechanisms by which RA protects against cognitive impairment associated with endotoxin-induced neuronal injury.

Data Sharing Statement

All data needed to evaluate the conclusions in the paper are present in the paper.

Acknowledgments

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest.

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