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Original Article

Lactobacillus paracasei effectively protects neuronal function in severe traumatic brain injury in rats by suppressing systemic inflammation

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Abstract: Objectives: Traumatic brain injury (TBI) is a growing public health concern with a high mortality rate. Our previous study identified *Lactobacillus paracasei* L9 (LP) as a promising neuroprotective agent. This study aimed to investigate the changes in neural function and gut barrier function in TBI rats after LP intervention and elucidate its protective role and mechanism in TBI. Methods: A rat model of severe TBI was established based on weight loss. Neurological function score, immunofluorescence staining of $NeuN^+$, $Iba\text{-}1^+$, and $CD68^+$ cells in brain tissue, dry-wet weight method for brain water content, Evans blue for blood-brain barrier permeability, real-time qPCR for occludin, Zonula Occludens-1 (ZO-1), intercellular Adhesion Molecule-1 (ICAM-1), toll-like receptor 4 (TLR4)/Myeloid differentiation primary response 88 (Myd88) expression in brain and intestinal tissue, and ELISA for inflammatory factors, LPS, and flagellin expression were evaluated at 1, 3, and 7 days post-injury. Results: LP administration significantly improved behavioral performance on days 3 and 7 post-TBI, increased $NeuN^+$ cells, decreased $Iba\text{-}1^+$ and $CD68^+$ cells, reduced brain edema, decreased Evans blue content, enhanced tight junction proteins in the damaged cortex, and reduced inflammatory factor expression. In the intestine, LP intervention improved villus height and crypt depth, upregulated occludin and ZO-1 mRNA expression, downregulated ICAM-1, and reduced the serum levels of D-Lac and LPS. In addition, the LP group exhibited lower TLR4 and Myd88 expression levels in the brain. Conclusions: LP protects against TBI-induced neuronal dysfunction by restoring the gut barrier, suppressing systemic inflammation, and inhibiting cortical TLR4/Myd88, supporting its potential as an adjuvant therapy.

Keywords: Traumatic brain injury, lactobacillus paracasei l9, systemic inflammation, blood-brain barrier

Introduction

The incidence of traumatic brain injury (TBI) has increased drastically in recent years, making it a significant public health concern owing to its high mortality rate [1]. Apart from primary brain damage inflicted by acute trauma, secondary extracranial non-neural organ injury is also an important contributor to the elevated mortality rate. Although numerous basic and preclinical TBI studies have been conducted, the clinical efficacy of most interventions remains suboptimal. The quest for an effective treatment of TBI continues.

Probiotics have garnered attention owing to their therapeutic potential in Central Nervous System (CNS) diseases. Lactobacillus exhibits therapeutic effects on various neuropsychiatric disorders, possibly through regulation of the gut microbiota regulation [2-4]. For instance, *Lactobacillus reuteri* modulates neuroinflammation through metabolites when combined with a ketogenic diet, alleviating repetitive mild traumatic brain injury (mTBI)-induced impairments in adolescent mice [2]; *Lactobacillus paracasei* itself regulates intestinal flora structure and arginine metabolism to affect the progression of experimental autoimmune neuritis [3]; *Lactobacillus delbrueckii* inhibits toll-like receptor 4 (TLR4) signaling to reduce depression-like behavior in mice [4]. However, these studies focused on chronic neuropsychiatric conditions or mild brain injuries, with mechanisms centered on metabolism regulation or direct TLR4 inhibition, while the role of *Lactobacillus* in post-acute severe neural trauma (e.g., severe TBI) is understudied. This constitutes the first novelty of our study, as we target

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severe TBI, a scenario rarely explored in previous Lactobacillus-related neuroprotection research. Animal models indicate that TBI induces structural and functional alterations in the gut within hours of post-injury, including villus damage, increased barrier permeability, and intestinal dysmotility [5, 6]. These changes can lead to gastrointestinal symptoms such as vomiting, bloating, and constipation. Moreover, gastrointestinal dysfunction post-TBI can exacerbate the severity of secondary brain injury and affect patient prognosis [7, 8]. Notably, systemic inflammation modulates CNS inflammation in TBI. Hanscom et al. (2021) demonstrated that acute colitis during chronic experimental TBI in mice induces persistent systemic inflammation, which further exacerbates CNS inflammation and neurological deficits by disrupting the gut-brain axis [9]. El Baassiri et al. (2024) found that Ccr2-dependent monocytes, activated by systemic inflammation post-TBI, infiltrate the gut to exacerbate intestinal inflammation and indirectly modulate CNS inflammation through gut serotonergic signaling [10]. Wei et al. (2023) reported that chronic alcoholic systemic inflammation disrupts the microbiome-gut-brain axis, promoting microglia-mediated neuroinflammation in the CNS, indicating that systemic inflammation can trigger CNS inflammation via similar axis-dependent mechanisms across different pathological conditions [11]. Clinically, Greene et al. (2024) observed that sustained systemic inflammation in long COVID patients leads to blood-brain barrier (BBB) disruption, allowing peripheral inflammatory factors to enter the CNS and induce cognitive impairment-related neuroinflammation [12]. The pivotal role of probiotics in gut microbiota regulation and gut structure and function may mitigate central nervous system damage via the gut-brain axis [13]. Our study further links gut barrier restoration to systemic inflammation suppression and subsequent neuroprotection (rather than focusing solely on metabolism or individual signaling pathways), which is the second novelty compared to previous reports.

Our previous study identified *Lactobacillus paracasei* L9 (LP) as a promising neuroprotective agent. We propose that LP can restore gut microbiota dysbiosis post-TBI, thereby influencing the gut-brain axis to promote brain injury recovery. This study aims to elucidate the changes in neural function and gut barrier function in TBI rats after LP intervention, investigate whether LP can improve TBI rat neural function via the gut-brain axis, further elucidate the protective role and mechanism of probiotics in TBI (by systematically verifying central and peripheral indicators), and provide novel strategies for TBI treatment; this systematic verification of central (brain edema, BBB permeability) and peripheral (intestinal villus structure, serum inflammatory factors) indicators establishes a more comprehensive gut-brain axis regulatory framework, which is the third novelty of our study.

Material and methods

LP preparation

A suitable quantity of LP (a gift from the Department of Microbiology, Central South University) was grown anaerobically in Rogosa and Sharpe (MRS) liquid medium (Haibo Biotechnology, Qingdao, China) for 48 h. The bacterial suspension was measured using a spectrophotometer, aliquoted, and stored at -80°C. Prior to use, the bacteria were centrifuged at 4°C, 5,000 g for 15 min, resuspended in physiological saline, and adjusted to a final concentration of 2×10^{10} colony-forming units (CFU)/ml.

TBI animal model

A total of 45 male specific pathogen-free (SPF)-grade SD rats (6- to 8-weeks-old, 250-300 g) were purchased from Beijing Vital River Laboratory Animal Technology Co. and kept under standard conditions in our local animal facility in pathogen-free cages with food and water available ad libitum. All experimental procedures adhered to the guidelines of the Animal Ethics Committee of Nanchang First Hospital. Rats were anesthetized with 3% pentobarbital at a dose of 50 mg/kg via intraperitoneal injection. After successful anesthesia, the skull was secured on a stereotaxic apparatus, incised above the cranium, and the sagittal suture point was identified. A 5 mm diameter bone window was drilled centered on the sagittal suture point, 1.5 mm posterior and 2.5 mm right. A 40 g weight was dropped vertically from 25 cm to induce severe TBI. The skin was sutured, and the rat was returned to its cage post-anesthesia, provided with ample food and water, and maintained under normal condi-

tions. The control group underwent craniotomy without TBI.

The 45 male SD rats (250-300 g) were randomly divided into three groups, each with 15 rats, including the sham, TBI, and TBI+LP groups. Rats in the TBI+LP group received 0.5 ml LP suspension (1×10^{10} CFU) daily via gavage, while the other groups received saline (0.5 ml). The modified neurological severity score (mNSS) was assessed on days 1, 3, and 7 post-injury to evaluate cognitive function. Two individuals proficient in the mNSS evaluated each group and documented their scores. The scale included limb movement (six points), sensation (two points), beam balance (six points), reflexes, and abnormal movements (four points). The lowest score was 0 and the highest score was 18, with higher scores indicating more severe neural dysfunction [14].

Brain tissue slice

On days 1, 3, or 7 post-TBI, the rats were anesthetized with 3% pentobarbital sodium at a dose of 50 mg/kg via intraperitoneal injection (consistent with the anesthesia protocol for TBI model establishment). Successful anesthesia was confirmed by the loss of consciousness and absence of pain reflexes (e.g., paw withdrawal). The rats were then subjected to cardiac perfusion with 20 ml 0.01 M phosphate-buffered saline (PBS) followed by 20 ml 4% paraformaldehyde. Euthanasia was verified by the cessation of heartbeat and breathing, ensuring no residual vital signs before subsequent tissue collection. After perfusion, the brain was extracted, immersed in 4% paraformaldehyde for 2 days at 4°C, embedded in optimal cutting temperature (OCT), and coronal brain slices were obtained.

Immunofluorescence staining

The brain sections were treated with 3% hydrogen peroxide (H_2O_2) solution at 37°C for 20 min, washed with PBS, and incubated in 0.5% Triton solution at 37°C for 2 h. Subsequently, the sections were incubated with rabbit monoclonal anti-NeuN antibody (1:250, ab177487), rabbit monoclonal anti-Iba1 antibody (1:200, ab178846), and rabbit monoclonal anti-CD68 antibody (1:200, ab283654) overnight at 4°C. Following washing with PBS, the sections were incubated with goat Anti-Rabbit IgG H&L (Cy3®) (1:500, ab6953) or goat anti-rabbit IgG H&L (Alexa Fluor® 488) (1:500, ab150077) in the dark. All antibodies were purchased from Abcam (Shanghai, China). 4',6-diamidino-2-phenylindole (DAPI) was used to stain the nucleus for 5 min, followed by fluorescent mounting media for sealing, and the results were observed under fluorescent microscopy. Three brain sections with identical locations from each sample were chosen, and the number of $NeuN^+$ neurons, $Iba\text{-}1^+$ microglia, and $CD68^+$ activated microglia within the five regions surrounding the lesion area were counted.

Evans blue detection of blood-brain barrier (BBB) permeability

Evans blue (Sigma, MO, USA) was injected into the tail veins of the rats. After 2 h of circulation, brain vessels were flushed with PBS (40-50 ml) via left ventricular perfusion. The cortex was peeled off and weighed into a 1.5 ml microcentrifuge tube (EP tube), 1 ml 50% trichloroacetic acid (TCA) solution was added, homogenized on a tissue grinder, and centrifuged at 3,000 g for 20 min at 4°C. The supernatant (30 μL) was mixed with 120 μL absolute ethanol. The absorbance was determined at 620 nm using an enzyme label analyzer. An Evans blue standard curve was constructed, the derived optical density (OD) values were substituted into the equation of the curve for calculation, and the concentration was converted to μg/g based on tissue weight.

Detection of cortical brain water content

The fresh brain was divided into the contralateral cortex (centered on the injury site, diameter 4.5 mm) and the ipsilateral cortex (corresponding position). Brain tissue was placed on tin foil and weighed. The brain tissue and foil were baked at 90°C for 72 h. Dry weight was measured after removal. Water content was calculated as 100 × (wet weight-dry weight)/wet weight.

ELISA

TNF-α and IL-1β expression levels in the damaged cortex and serum (Elabscience, Wuhan, China), D-lactate (Elabscience, Wuhan, China), LPS (Bioendo, Xiamen, China), and flagellin (CUSABIO, Wuhan, China) were measured using ELISA kits, according to the manufacturer's instructions.

Paraffin sectioning

Intestinal specimens were removed from 70% ethanol and placed in a paraffin embedding box. The thickness of the paraffin sections was set to 5 μm, and hematoxylin-eosin (HE) staining was used to detect small intestine morphology.

Real-time qPCR

Total RNA was extracted from cells and tissues using TRIzol reagent (Takara, Shanghai, China), and RNA purity and concentration were measured using an ultraviolet spectrophotometer. Total RNA was reverse transcribed to cDNA using PrimeScript™ RT reagent Kit (Takara, Shanghai, China), and occludin/ZO-1/ICAM-1/Tlr4/Myd88 expression levels were determined using real-time fluorescence quantitative PCR with 2×SYBR Green qPCR Master Mix (Takara, Shanghai, China). β-actin was used as an internal control. The reaction conditions were as follows: 95°C for 10 min; 40 cycles of 95°C for 15 s; 60°C for 60 s. Three experiments were performed to calculate relative expression levels using the $2^{-\Delta\Delta Ct}$ method. Primers were synthesized by Shanghai Shenergy Biotechnology Co., Ltd.

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Statistical analyses were performed using SPSS 24.0. Single time-point data were analyzed using one-way ANOVA, and multiple time-point data were analyzed using two-way ANOVA. Multiple comparisons were performed using the Bonferroni method, and statistical significance was set at $P < 0.05$.

Results

LP ameliorates nerve function in TBI rats

To investigate the neuroprotective role of LP in TBI rats, we evaluated sensory motor function using mNSS scores on days 1, 3, and 7 post-injury. Lower NSS scores indicate better neural function. The results revealed the most severe neural function damage on day 1 post-injury, with a significant increase in mNSS scores in the model group but no improvement in the LP group. On day 3, there was a decrease in the model group's scores compared to those on day 1. However, the mNSS score of the LP group rats was significantly lower than that of the model group. The model group's scores further declined by day 7, with a distinct decrease in the LP group's scores when compared to the model group. These results suggest that LP administration improved the behavioral performance of injured rats on days 3 and 7 post-injury (**Figure 1A**). By immunofluorescence staining, we labeled $NeuN^+$ cells in the brain tissue, showing a consistent trend among all groups at days 3 and 7 post-TBI, with a significant decrease in $NeuN^+$ cells around the damaged cortex in the model group. LP treatment significantly increased the number of $NeuN^+$ cells in the TBI group (**Figure 1B**, **1C**).

LP ameliorates BBB in TBI rats

TBI can induce brain edema, leading to increased intracranial pressure and further exacerbating brain injury, which seriously affects disease prognosis. We detected the water content in the brain tissue using the dry-wet weight method, reflecting brain edema in the damaged cortex. Compared to the control group, brain water content in TBI group rats significantly increased on day 3 post-injury, while it significantly decreased in the LP group rats. These results indicate that TBI rats developed severe brain edema on days 3 and 7 post-injury, and LP intervention alleviated edema severity (**Figure 2A**). We injected Evans Blue solution into rat blood via the tail vein, and after circulation, we detected the amount of dye penetrating the brain tissue to determine BBB permeability. We found that the Evans Blue content in the brain tissue of the TBI group on days 3 and 7 post-injury significantly increased compared to the control group. The Evans blue content in the brain tissue of the LP group significantly decreased. Based on these results, LP ameliorated brain edema after TBI, potentially owing to a reduction in BBB permeability post-injury (**Figure 2B**). Further measurement of occludin and ZO-1 mRNA levels revealed that occludin and ZO-1 mRNA expression levels in the injured cortex tissues of the TBI group were significantly lower than those in the control group, and the expression of occludin and ZO-1 mRNA in the LP group was significantly higher than that in the model group (**Figure 2C**). These results sug-

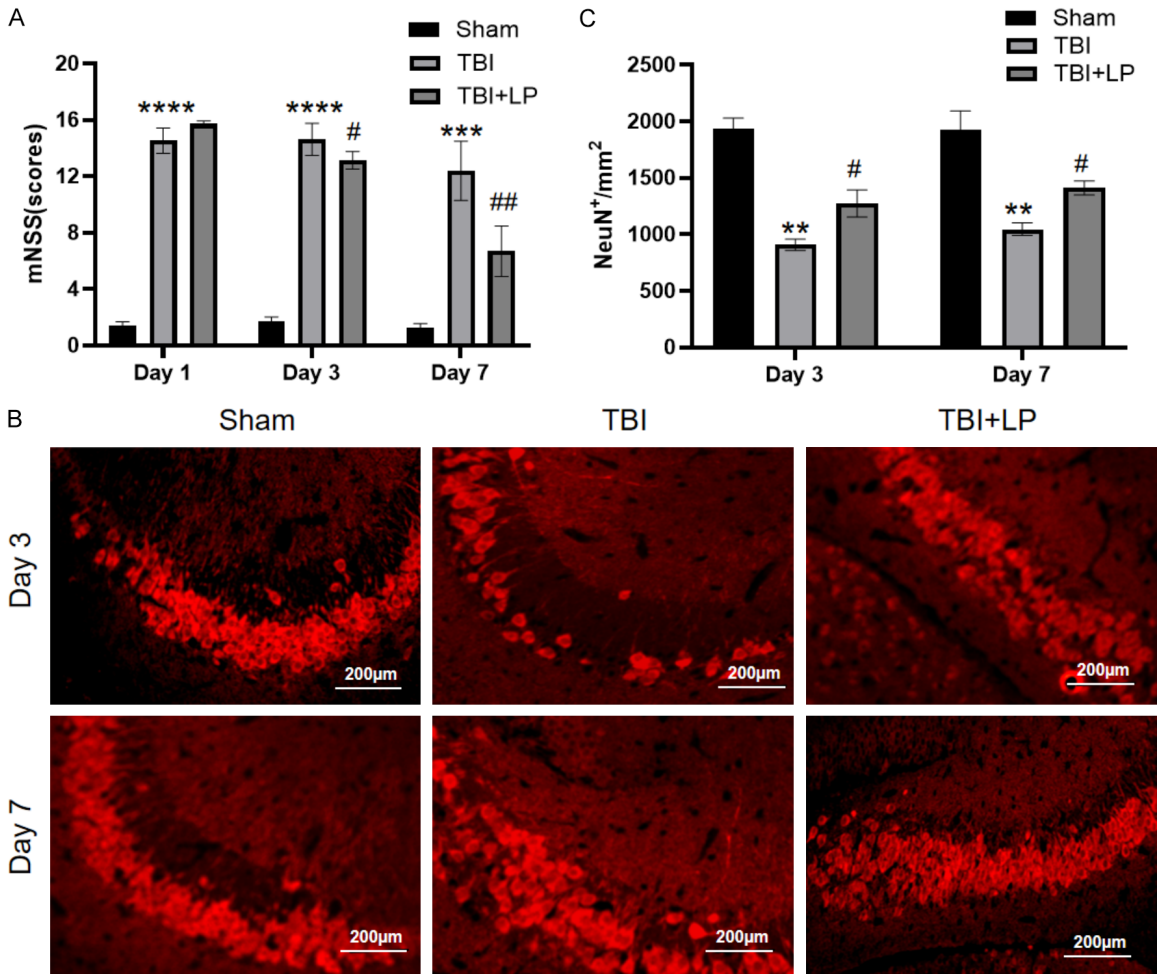

Figure 1. LP ameliorates nerve function in TBI (n = 5). A. Modified neurological severity score (mNSS) score evaluation of rat sensory motor function. Two-way repeated-measures ANOVA to test main effects of group, time, and their interaction. Post-hoc multiple comparisons were conducted using the Bonferroni method to compare mNSS scores between groups at each time point. B. Immunofluorescence staining for NeuN$^+$ cells in damaged cortex (scale bar is 100 μm). C. Statistical analysis of NeuN$^+$ cells. For each time point, one-way ANOVA was used to compare cell counts among the 3 groups. Bonferroni post-hoc tests were applied to identify pairwise differences. **P < 0.01, ****P < 0.0001 vs. Sham; #P < 0.05, ##P < 0.01 vs. TBI.

gest that LP can alleviate the degree of brain edema and BBB permeability in TBI rats, which may be related to its ability to enhance the expression of tight junction proteins in the damaged cortex tissues.

LP reduces brain inflammation in TBI rats

Inflammation is a key factor in secondary brain damage in TBI [15]. Microglia are pivotal cells that initiate inflammatory responses in the CNS [16]. Using immunohistochemistry, we labeled microglia expressing Iba-1. The results indicated a significant increase in the number of Iba-1$^+$ cells in the TBI group on days 3 and 7 post-injury, with a significant decrease in the LP group compared with the TBI group (**Figure 3A**, **3B**). To further elucidate the effect of LP on microglial activation in the cortex surrounding the lesion in TBI rats, CD68 expressing cells were labeled using immunofluorescence staining. The results showed minimal CD68$^+$ cells in the rat cortex under normal conditions, with a significant increase on day 3 post-injury in the cortex surrounding the lesion in the TBI group, whereas on day 7, the number of CD68$^+$ cells remained elevated. The LP group showed a significant reduction in the number of CD68$^+$ cells compared to the TBI group (**Figure 3A**, **3B**). This confirmed that LP could reduce the number of activated microglia in the cortex surrounding

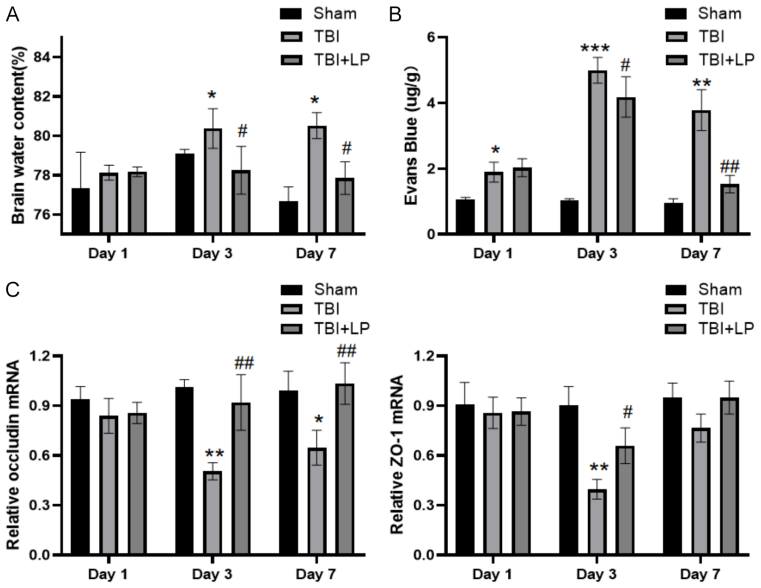

Figure 2. LP ameliorates Blood-Brain Barrier (BBB) in TBI (n = 5). A. Dry-wet weight method detects brain edema severity in rats' damaged cortex. B. Evans Blue method measures the permeability of the blood brain barrier in rats' damaged cortex. C. Real-time qPCR evaluates the expression of occludin and ZO-1 mRNA. One-way ANOVA was performed for each time point to compare brain water content among groups. Bonferroni post-hoc tests were used to determine significant differences between groups. *P < 0.05, **P < 0.01, ****P < 0.001 vs. Sham; #P < 0.05, ##P < 0.01 vs. TBI.

the lesion in TBI rats on days 3 and 7 post-injury.

Research indicates that TBI can elevate the expression levels of inflammatory factors, including TNF-α and IL-1β, at the injury site. Moreover, excessive accumulation of these brain-derived inflammatory factors exacerbates neural damage after TBI [17]. We used to measure the expression levels of inflammatory factors in the rat cortex. The results showed that TNF-α expression levels significantly increased compared to the control group on days 3 and 7 post-TBI, whereas in the TBI group, the expression level of TNF-α in the cortex of the TBI+LP group rats decreased notably (**Figure 3C**). The expression level of IL-1β in the TBI group also significantly increased compared to the control group on days 3 and 7 post-injury, with the expression level in the cortex of the TBI+LP group rats significantly declining on days 3 and 7 post-injury (**Figure 3D**). These findings suggest that LP can potentially lower the expression levels of inflammatory factors in the damaged cortex of TBI rats on days 3 and 7 post-injury.

LP ameliorates colon mucosa structure and permeability in TBI rats

To verify LP colonization in the gut of TBI model rats, we evaluated LP levels using quantitative real-time PCR after scraping the intestinal mucosal layer. There was no significant difference in LP concentration between the TBI and Sham groups post-TBI on days 3 and 7. Post-LP administration to TBI rats, the LP level in the small intestine significantly increased (**Figure 4A**), suggesting that LP can inhabit the small intestinal mucosa after LP administration. Hematoxylin and eosin (HE) staining was used to analyze the morphology of the small intestinal mucosa. On Day 3 post-TBI, there was a notable reduction in villus height and crypt depth in the TBI group compared to those in the LP group (**Figure 4B**). On Day 7 post-TBI, villus height and crypt depth remained significantly damaged; however, LP intervention significantly improved these parameters (**Figure 4B**). The D-Lac level in the blood is an indicator of gut mucosal permeability. The higher the D-Lac content, the greater the barrier permeability. On days 3 and 7 post-injury, the D-Lac content in the serum of TBI rats significantly increased, indicating a significant increase in gut mucosal permeability, whereas in the TBI group, the D-Lac content in the serum of TBI+LP group rats significantly decreased (**Figure 4C**). The expression of the tight junction proteins occludin and ZO-1 in the small intestine was detected using real-time qPCR. The results showed that the mRNA expression of occludin and ZO-1 in the small intestine of TBI rats significantly decreased on days 3 and 7 post-TBI, whereas in the TBI group, the mRNA expression of occludin and ZO-1 in the small intestine of TBI+LP group rats significantly increased. Conversely, ICAM-1 expression in the small intestinal epithelial cells of TBI rats significantly increased on days 3 and 7 post-injury, which was downregulated post LP intervention (**Figure 4D**). These findings suggest that LP can

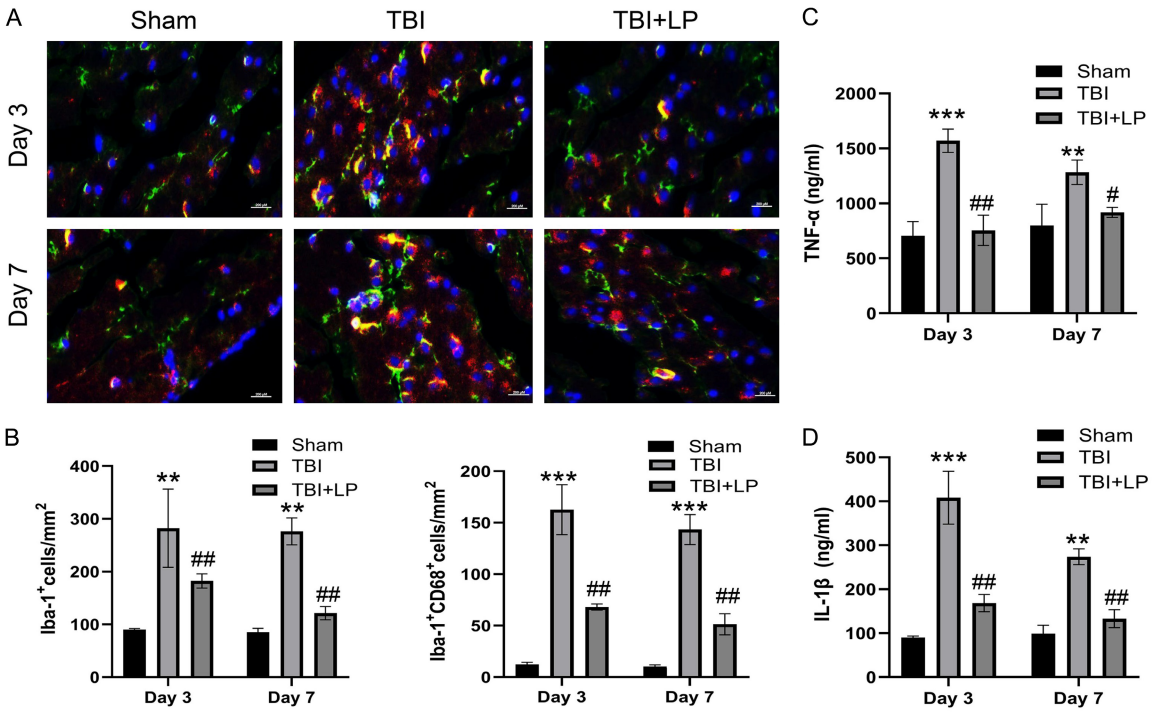

Figure 3. LP ameliorates brain inflammation (n = 5). A. Double immunofluorescence staining of microglial activation (scale bar=100 μm; Green: Iba-1 (microglia marker); Red: CD68 (activated microglia marker); Blue: DAPI (nucleus marker)); B. Statistics of Iba-1$^+$ and Iba-1$^+$ CD68$^+$ cells; C. Lesion cortex TNF-α expression level; D. Lesion cortex IL-1β expression level. For each time point, one-way ANOVA was used to compare cell counts among the 3 groups. Bonferroni post-hoc tests were applied to identify pairwise differences. **P < 0.01, ***P < 0.001 versus Sham; #P < 0.05, ##P < 0.01 versus TBI.

reduce post-injury gut barrier permeability on days three and seven post-TBI.

LP ameliorates systemic inflammation in TBI rats

Systemic inflammation potentially mediates secondary brain injury [18]. Intestinal microbiota is primarily composed of Gram-negative and Gram-positive bacteria. Dysbiosis of the gut flora, accompanied by elevated intestinal barrier permeability, can cause gram-negative bacteria to enter the bloodstream, leading to an increase in circulatory LPS. However, Gram-positive bacteria entering the blood cause an elevation in flagellin levels. We detected the levels of LPS and flagellin in the blood of rats in various groups using ELISA kits, and the results indicated that after injury, the TBI group exhibited significantly elevated blood LPS content on day 3, whereas LP treatment reduced the level of LPS in rats. Similar changes were observed on day 7 (**Figure 5A**). No significant change was observed in blood flagellin content among all groups on days 3 and 7 (**Figure 5B**). Subsequently, we measured the levels of the inflammatory factors TNF-α and IL-1β in the blood of the rats in each group using ELISA. On day 3 post-injury, the TNF-α content in the TBI group was significantly higher than that in the sham group, but it decreased significantly in the TBI+LP group compared with the TBI group. On day 7, the TNF-α content in the TBI group decreased compared with that on day 3, and LP administration further decreased the TNF-α content in the TBI group (**Figure 5C**). Similarly, on days 3 and 7, IL-1β levels in the TBI group increased significantly, but LP intervention significantly decreased these levels (**Figure 5D**). Toll-like receptors (TLRs) play pivotal roles in regulating inflammation. In a TBI animal model, TLR4 expression levels were significantly increased post-injury [15]. To further determine the effect of LP on TLR4/Myd88 expression in the brain tissue of TBI rat models, we used real-time qPCR to detect the mRNA expression levels of TLR4 and its associated molecule, Myd88, in the damaged cortex. The results showed that on days 3 and 7 post-injury, TLR4

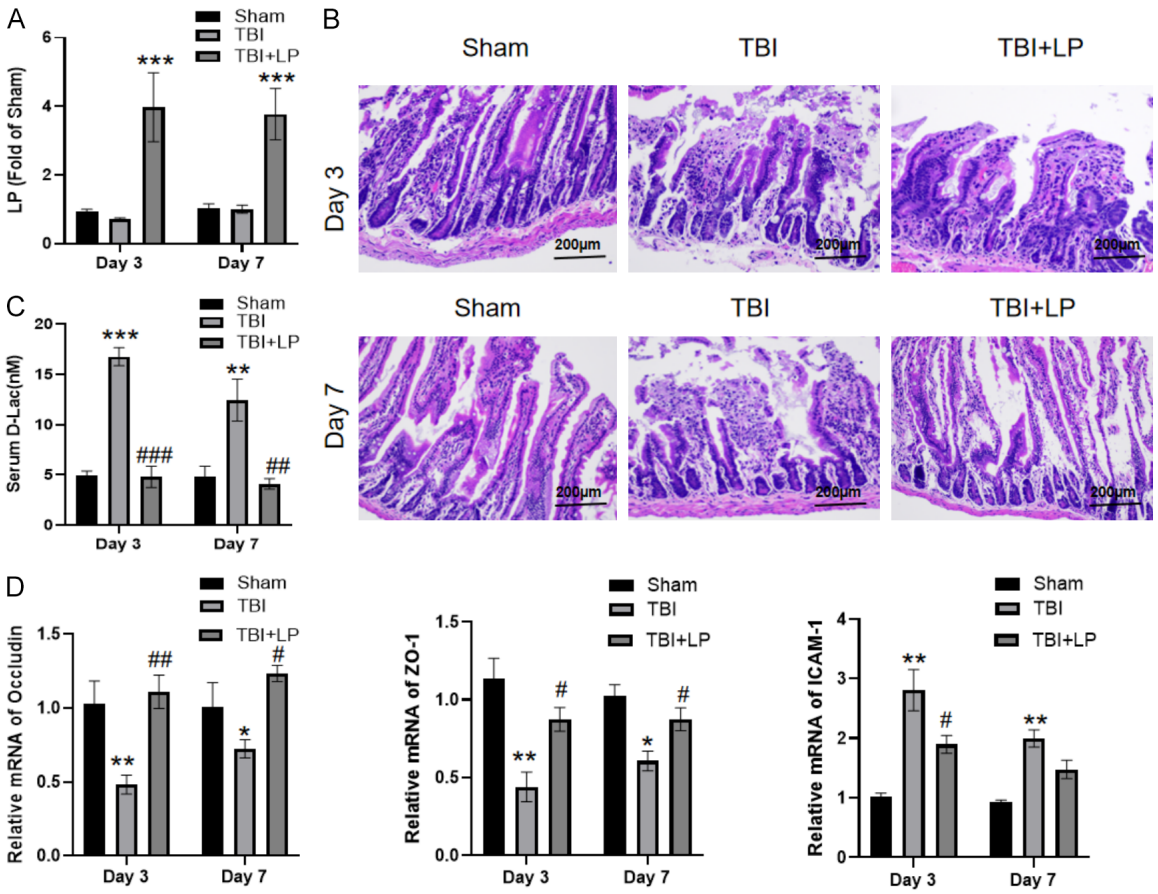

Figure 4. LP ameliorates gut mucosal structure and permeability in TBI rats (n = 5). A. qPCR detection of LP colonization in rat small intestine; B. HE staining of small intestinal mucosa morphology on Day 3 and 7 post-TBI (scale bar = 200 μm); C. ELISA measurement of serum D-lactic levels; D. Real-time qPCR detection of occludin, ZO-1, and ICAM-1 expression in rat small intestine. One-way ANOVA was performed for each analyte and time point to compare concentrations among groups. Bonferroni post-hoc tests were used to identify significant pairwise group differences. *P < 0.05, **P < 0.01, ***P < 0.001 vs. Sham; #P < 0.05, ##P < 0.01 vs. TBI.

mRNA expression levels in the damaged cortex of the TBI group were significantly higher than those in the sham group, but LP treatment significantly decreased TLR4 mRNA expression levels in rats (**Figure 5E**). As a molecule associated with TLR4, Myd88 mRNA expression levels followed a similar trend. On day 3, Myd88 mRNA expression levels in the TBI group were significantly higher than those in the sham group, but LP administration significantly decreased these levels (**Figure 5F**). These results suggest that LP has an inhibitory effect on the upregulation of TLR4/Myd88 expression levels in the damaged cortex after TBI.

Discussion

On post-injury days 1, 3, and 7, all groups demonstrated neurological deficits, with a profound behavioral decline on day 1. However, LP's protective effects were not observed on day 1 post-TBI, but post-LP intervention ameliorated behavioral outcomes on post-injury days 3 and 7, suggesting effective neurological recovery post-LP intervention after TBI. LP functions by increasing the number of viable neurons around areas of brain damage induced by TBI, reducing brain water content and BBB permeability, and suppressing microglial activation. Research has indicated that probiotics exhibit neuroinflammatory suppression. Milk fermented by *Lactobacillus paracasei* can reduce LPS-induced microglial activation and improve learning and memory functions in vivo and in vitro [19]. Probiotics alleviate anxiety and depression in chronically stressed rats, potentially by regulating neuroinflammation and reducing TNF-α and IFN-γ expression in the hippocampus [20]. In

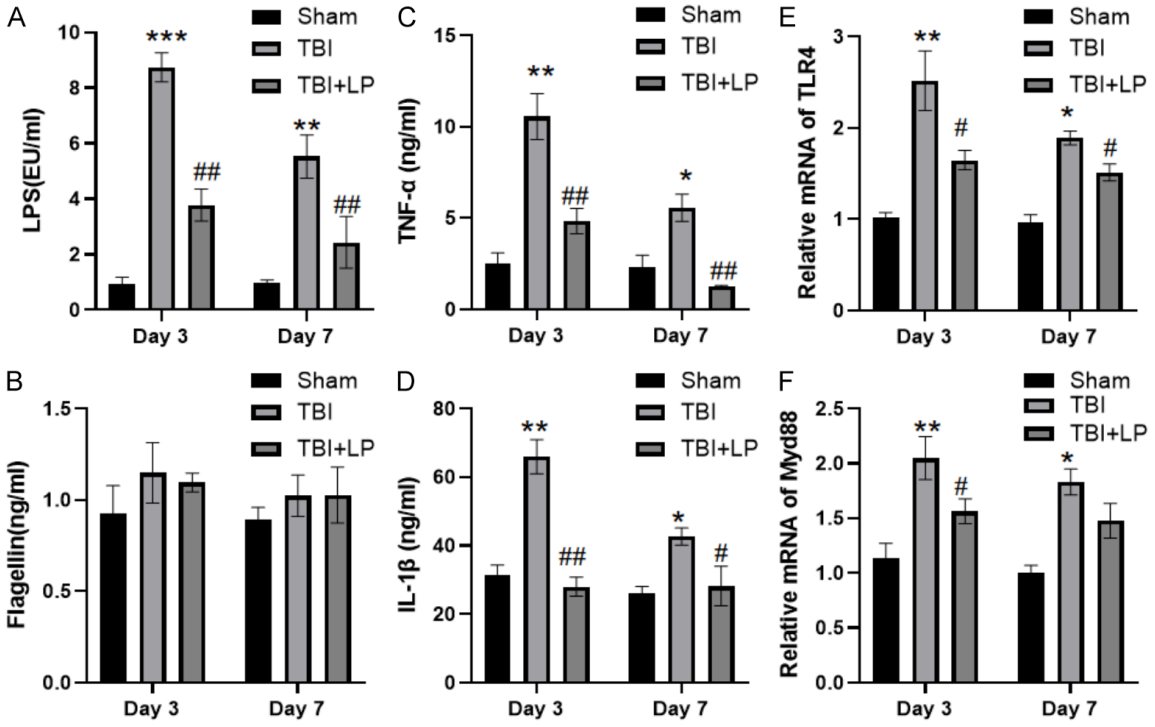

Figure 5. LP Ameliorates Systemic Inflammation (n = 5). A. LPS levels in serum; B. Flagellin level in serum. C. ELISA evaluating TNF-α level in serum; D. ELISA measuring IL-1β level in serum. E. Real-time qPCR detecting TLR4 mRNA expression level; F. Real-time qPCR assessing Myd88 mRNA expression level. One-way ANOVA was performed for each analyte and time point to compare concentrations among groups. Bonferroni post-hoc tests were used to identify significant pairwise group differences. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. Sham; #P < 0.05, ##P < 0.01 vs. TBI.

this study, LP administration significantly reduced the total number of activated microglia around the damaged area in rats with TBI. Moreover, the expression levels of TNF-α and IL-1β in the injured cortex significantly decreased post-LP administration. This suggests that LP also plays a role in suppressing acute neural trauma-induced neuroinflammation. Given the pivotal role of neuroinflammation in secondary brain injury progression and LP's inhibitory effect of LP on inflammation in TBI rat brain tissue, one possible mechanism for LP's neuroprotective effect of LP post-TBI is its involvement in regulating neuroinflammatory levels post-TBI.

The question remains of how probiotics enhance nervous system function via gut colonization. The progression of TBI is complex, causing CNS damage and non-neural effects, including in the gastrointestinal system [9]. Post-injury bowel function disturbances manifest as intestinal motility disorders and disruption of barrier integrity. TBI leads to significant changes in villus height and surface area in the rat small intestine, and crypt depth reduction. Our study using HE staining revealed a marked alteration on day 3 post-injury in the small intestinal mucosal structure in TBI rats, including reduced villus height and crypt depth. Intestinal barrier dysfunction can induce bacterial translocation into the bloodstream, triggering bacteremia and systemic inflammation, potentially leading to multiple organ dysfunction syndrome (MODS). Our results showed a significant increase in D-Lac and LPS levels in TBI rat serum post-injury, indicating increased intestinal barrier permeability, which was mitigated by LP intervention. These findings collectively suggest that LP may strengthen intestinal barrier integrity post-TBI, potentially serving as a neuroprotective mechanism, including LP-released outer membrane vesicles (OMVs), which contain proteins/lipids to enhance tight junction protein expression, or metabolites (e.g., short-chain fatty acids [SCFAs] that promote intestinal mucosal repair), although direct experimental evidence needs supplementation.

Systemic inflammation also plays a role in the bidirectional regulation of the gut-brain axis induced by TBI [10, 19]. Clinical studies have reported cognitive impairment and reduced quality of life in patients with severe systemic inflammation [20]. Animal studies have indicated an increase in LPS levels in the blood and brain inflammation after intestinal barrier damage, resulting in behavioral abnormalities, such as anxiety or memory loss [21]. Our study also found elevated LPS and inflammatory cytokine levels in TBI rat blood on day 3 post-injury, along with a significant increase in TLR4 and Myd88 mRNA expression in the damaged cortex. It should be noted that the reduction of inflammation solely by TLR4 and Myd88 expression is incomplete, as infiltrating myeloid cells in the brain may affect their mRNA levels; however, LP may indirectly regulate these molecules by reducing peripheral inflammatory stimuli (e.g., LPS). TBI disrupts BBB integrity and facilitates LPS entry into damaged brain tissue. These findings suggest that increased intestinal barrier permeability post-TBI can exacerbate neuroinflammatory responses by increasing peripheral blood LPS levels. In this study, LP intervention in TBI rats restored the intestinal barrier integrity, decreased serum LPS levels, and suppressed TLR4/Myd88 expression in the damaged cortex. Collectively, these results indicate that LP's protective effect of LP on TBI neurological function is related to reduced intestinal barrier permeability and circulating LPS levels and inhibition of TLR4/Myd88 expression in damaged tissues.

This study detected LP levels in the mucosa of the small intestine using quantitative real-time PCR. The results showed no significant difference in LP concentration between the TBI and sham groups on days 3 and 7 post-injury, indicating that TBI itself does not reduce intestinal LP levels. However, TBI may cause broader intestinal microbiota dysbiosis. Rahman et al. (2024) used machine learning models to confirm that controlled cortical impact (a TBI model) alters gut integrity, which is often accompanied by changes in the abundance of gut flora such as Akkermansia muciniphila [5]. DeSana et al. (2024) found that acute TBI-induced intestinal hypoxia leads to a bloom in Akkermansia muciniphila, disrupting the balance of the intestinal microbiota [6]. Although this study did not comprehensively profile the intestinal microbiota, these indirect findings suggest that TBI induces intestinal microbiota dysbiosis, even though it does not specifically reduce LP levels.

In conclusion, TBI can lead to structural damage of the intestinal mucosa, which can be improved by LP intervention. LP inhibits systemic inflammation and TLR4/Myd88 expression in the damaged cortex, and thereby, neuroinflammation. This study provides a rationale for future LP adjuvant therapy in TBI patients.

Disclosure of conflict of interest

None.

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