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Original Research

Dexmedetomidine Protects the Brain: Exploring the α2AR/FAK Pathway in Post-Stroke Intestinal Barrier Repair

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Abstract

Introduction: Globally, ischemic stroke is a major cause of mortality and disability, posing a significant challenge in clinical practice and public health. Recent studies have reported that stroke leads to the impairment of the intestinal barrier and the migration of intestinal bacteria to multiple organs. This process exacerbates neurological damage by further impairing intestinal barrier function and leading to bacterial translocation. Dexmedetomidine (Dex), an α2-adrenoceptor (α2AR) agonist, has proven anti-cerebral ischemic effects, yet its effects in post-stroke intestinal dysfunction remain unclear. This study aimed to determine whether Dex mitigates intestinal dysfunction and brain injury following cerebral ischemia-reperfusion. **Methods**: A C57BL/6J mouse model of middle cerebral artery occlusion (MCAO) was used for *in vivo* experiments, while lipopolysaccharide (LPS)-induced Caco-2 monolayers served as an *in vitro* model of intestinal barrier dysfunction. Neuronal apoptosis was evaluated using neuronal nuclei (NeuN) and terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (TUNEL) double labeling. Reverse transcription-quantitative PCR (RT-qPCR) was performed to measure pro-inflammatory cytokines tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6. Intestinal permeability was assessed using histological score, serum fluorescein isothiocyanate (FITC)-dextran fluorescence, and endotoxin levels. The expression levels of epithelial cadherin (E-cadherin), zonula occludens-1 (ZO-1), and occludin were analyzed by western blot and immunofluorescence. Statistical analyses included analysis of variance with Tukey's post-hoc test. **Results**: Dex treatment significantly reduced cerebral infarct volume ($p < 0.001$) and improved neurological scores compared to MCAO controls. Neuronal apoptosis was significantly inhibited ($p < 0.01$), as evidenced by reduced TUNEL-positive cells in Dex-treatment MCAO mice. TNF-α, IL-1β and IL-6 were markedly downregulated ($p < 0.05$). While MCAO increased intestinal permeability (elevated serum FITC-dextran and endotoxin levels, $p < 0.01$), Dex treatment restored barrier integrity. Dex upregulated E-cadherin expression significantly ($p < 0.05$) but did not restore the decreased levels of ZO-1 and occludin following MCAO. Dex promoted intestinal permeability repair and alleviated brain injury via the α2AR/focal adhesion kinase (FAK) pathway in MCAO mice. Similarly, Dex mitigated LPS-induced barrier dysfunction in Caco-2 monolayers by restoring FAK expression and improving intestinal barrier integrity. **Conclusions**: Dex alleviates post-stroke intestinal barrier dysfunction and mitigates brain injury, possibly through activating the α2AR/FAK pathway. These findings underscore a potential therapeutic strategy for addressing secondary complications of ischemic stroke and improving patient outcomes.

Keywords: stroke; brain; intestinal barrier; alpha2-adrenoceptor; focal adhesion kinase

1. Introduction

Ischemic stroke (IS) is a major cause of mortality and disability globally, resulting from interrupted cerebral blood flow, which leads to brain tissue damage and functional impairment [1]. Current therapeutic strategies include restoring blood flow to the brain and mitigating neuronal damage through pharmacologic or biological methods, such as stem cell transplantation [2,3]. However, due to the complexity of the nervous system and the challenges of neuronal regeneration, effective clinical treatments remain limited [4]. Therefore, identifying novel therapeutic agents and understanding their mechanisms is of great clinical significance.

Pathophysiological mechanisms of IS are categorized into primary injury, caused by reduced blood flow, and secondary injury, which arises from immune responses and inflammation [5]. During the acute phase, infiltration of neutrophils and macrophages produces pro-inflammatory cytokines and reactive oxygen species (ROS), leading to blood-brain barrier dysfunction and cerebral edema [6]. Furthermore, cluster of differentiation (CD)8+ T lymphocytes and $\gamma\delta$T cells migrate to infarcted regions, exacerbating neuronal damage by releasing cytokines such as interleukin (IL)-1β, IL-17, and interferon (IFN)-γ [7–10].

As the body's largest immune organ, the intestinal tract plays a crucial role in regulating systemic immune responses. IS-induced intestinal barrier dysfunction increases intestinal permeability, facilitating bacterial translocation and systemic inflammation [11–13]. Post-stroke, gut-derived immune cells, such as $\gamma\delta$T cells, migrate to brain infarct regions and exacerbate neuroinflammation in the brain post-IS [14–16]. Notably, improving intestinal bar-

rier function, such as gut flora modulation, has reduced IS-induced brain injury [17–20]. Dexmedetomidine (Dex), an α2-adrenoreceptor (α2AR) agonist, has shown promising neuroprotective effects in IS models. Dex mitigates neuronal apoptosis, calcium influx, and oxidative stress via po via transient receptor potential melastatin 2 and transient receptor potential vanilloid 1 pathways and reduces neuroinflammation by inhibiting the toll-like receptor 4 pathway [21–26]. It also alleviates pulmonary microvascular hyperpermeability in renal injury models through the α2AR/focal adhesion kinase (FAK) pathway [27]. However, whether Dex can repair IS-induced intestinal barrier dysfunction and its connection to brain injury remains unclear.

FAK, a cytoplasmic protein tyrosine kinase, plays a critical role in repairing damaged intestinal epithelial cells by modulating tight junctions (TJs) and adherens junctions (AJs) [28–30]. The previous study has shown that Dex regulates vascular permeability in injury models through the α2AR/FAK pathway [31]. However, its role in mitigating IS-induced brain injury by improving intestinal barrier function via the α2AR/FAK pathway remains to be explored.

This study aimed to investigate whether Dex ameliorates brain injury by repairing IS-induced intestinal barrier dysregulation and to explore the involvement of the α2AR/FAK signaling pathway using a mouse middle cerebral artery occlusion (MCAO) model and lipopolysaccharide (LPS)-induced Caco-2 monolayers.

2. Materials and Methods

2.1 Experimental Animals

Male C57BL/6 mice (20–25 g, 8–10 weeks) (Vital River Laboratory, Beijing, China) were housed in standardized conditions: temperature of 22 $\pm$ 2 °C, humidity of 65 $\pm$ 5%, and a 12-h light-dark cycle with ad libitum access to food and water. The approval for animal experimental research (approval code: 2022111229001) for this study was granted by the Medical Ethics Committee of the First Affiliated Hospital of University of South China. Animal handling procedures were in complete compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2 Transient MCAO Procedure

The MCAO model was established following Longa's method [32]. Mice were anesthetized using 5% isoflurane for induction and 1.5% for maintenance. The left common, internal, and external carotid arteries were carefully separated from the surrounding tissue. A silicone-coated nylon monofilament (RWD Life Science, Shenzhen, China) was inserted into the right internal carotid artery through the stump of the external carotid artery to occlude the origin of the middle cerebral artery, blocking distal blood flow. After 60 minutes, the monofilament was slowly withdrawn to restore blood flow. The incision was closed with 4-0 silk sutures, and body temperature was maintained at 37 $\pm$ 0.5 °C throughout the procedure. Sham-operated mice underwent the same surgical procedure without monofilament insertion.

2.3 Experimental Grouping

To evaluate the effect of Dex on MCAO-induced cerebral infarction and intestinal dysfunction, mice were divided into the following groups: sham (n = 5), MCAO (n = 5), and MCAO + Dex (n = 5). In the MCAO + Dex group, Dex (100 µg/kg; #HY-12719; MedChemExpress, Shanghai, China) was administered intraperitoneally in two doses, 12 hours apart, based on a previous study [33].

To investigate whether Dex ameliorates cerebral infarction and intestinal dysfunction via the α2AR/FAK pathway, mice were further divided into these subgroups: sham; MCAO; MCAO + Dex; MCAO + Dex + SKF 86466: SKF 86466 (1 mg/kg; #T23366; Targetmol, Shanghai, China) was administered intraperitoneally 10 min before Dex; and MCAO + Dex + BI 853520: BI 853520 (12.5 mg/kg; #T64167; Targetmol, Shanghai, China) was injected intraperitoneally 10 min prior to Dex. DMSO was used as the vehicle for SKF 86466 and BI 853520.

2.4 Triphenyl Tetrazolium Chloride (TTC) Staining

Mice were euthanized with cervical dislocation 24 hours after Dex treatment, and the brains were sectioned into 2-mm slices. Slices were immersed in 2% TTC solution (#17779; Sigma-Aldrich, St. Louis, MO, USA) at 37 °C for 30 min in the dark to assess the infarct volume [34]. After image capture, infarcted brain regions were quantified using ImageJ 1.48v software (NIH, Bethesda, MD, USA).

2.5 Neurological Function Assessment

Neurological deficits were assessed 24 hours post-treatment using Bederson's scale (0–5 points) [35,36]: 5, moribund or comatose; 4, severe spinning; 3, consistently strong and immediate circling; 2, mild consistent circling; 1, mild circling behavior; and 0, no observable deficit.

2.6 Double Immunofluorescence (IF) Staining

Brain sections (6 µm) were permeabilized using 0.1% Triton X-100 (50 µL, Beyotime, Shanghai, China), then incubated in sodium citrate-repair buffer (Beyotime). TUNEL reaction mixture (Yeasen, Shanghai, China) was applied for 60 min, followed by blocking with 1% BSA for 30 min. Sections were incubated with anti-NeuN primary antibody (1:1000, #ab104224; Abcam, Cambridge, MA, USA) and secondary antibody (1/1000, #ab150115; Abcam) [37]. Nuclei were counterstained with DAPI (Yeasen), and images were captured using a fluorescence microscope (BX51; Olympus, Tokyo, Japan) and analyzed with ImageJ.

2.7 Reverse Transcription-quantitative PCR (RT-qPCR)

Total RNA was extracted from brain tissues using the Total RNA Extraction Kit (#R1200; Solarbio, Beijing, China) following the manufacturer's protocol. Complementary DNA was synthesized using the PrimeScript™ RT reagent Kit (#RR047A; Takara, Otsu, Japan). RT-qPCR reaction was performed with TB Green® Premix Ex Taq™ II (#RR820A; Takara) and gene-specific primers (**Supplementary Table 1**). Relative mRNA levels were quantified using the $2^{-\Delta\Delta Ct}$ method and normalized to glyceraldehyde 3-phosphate dehydrogenase.

2.8 Hematoxylin-eosin (HE) Staining

Small intestine specimens were sectioned into 6-µm slices and stained with HE for light microscopy. Mucosal injury was assessed using established grading criteria [38]: 0, Normal mucosal villi; 1, Subepithelial gap at villus tips; 2, Dispersed epithelial degeneration at villus tips; 3, Apical degeneration, obtuse villi, and exposed lamina propria; 4, Shedding of epithelium, widened and shortened villi, exposed capillaries; and 5, Lamina propria disintegration, ulceration, and complete villus destruction.

2.9 Intestinal Permeability Analysis

Intestinal permeability was assessed using two methods. First, fluorescein isothiocyanate (FITC)-dextran 4000 (600 mg/kg body weight; #FD4, Sigma) via oral gavage following a 4-hour fast. Blood was collected, and serum fluorescence intensity was measured using a multimodal plate reader (excitation: 490 nm, emission: 520 nm; PerkinElmer EnSpire Plate Reader, Waltham, MA, USA).

Second, serum endotoxin concentrations were measured using the ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (#L00350, Genscript, Piscataway, NJ, USA) following the manufacturer's instructions.

2.10 Cell Culture

Human colonic epithelial Caco-2 cells (#CL-0050; Pricella Biotechnology Co., Ltd., Wuhan, China) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) - High Glucose (#SH30243.01B; Hyclone, Logan, UT, USA) supplemented with 10% fetal bovine serum (#HY-T1000; Sigma), 50 U/mL penicillin (Procell, Wuhan, China), and 50 U/mL streptomycin (Procell). Cells were maintained at 37 °C with 5% CO_2 in a humidified atmosphere. The cell line was validated by short tandem repeat (STR) profiling and tested negative for mycoplasma.

For barrier function assays, Caco-2 cells were seeded on 0.4 µm pore polyester membrane inserts (#3460; Corning, New York, NY, USA) in Transwell plates, with media changes every third day. LPS (1.0 ug/mL; #L2630; Sigma) was added to the basolateral side of Transwell monolayers for 24 hours to induce barrier dysfunction.

2.11 Western Blot

Total protein was extracted from small intestinal tissues and Caco-2 cells using a radioimmunoprecipitation assay (RIPA) buffer (#P0038; Beyotime, Shanghai, China), and protein concentrations were determined using the BCA Protein Assay Kit (#P0012; Beyotime). Equal protein amounts (15 µg per lane) were separated on 8–12% SDS-PAGE gels and transferred onto polyvinylidene difluoride (PVDF) membranes. Membranes were blocked with 5% non-fat milk, incubated overnight with primary antibodies at 4 °C, and then incubated with secondary antibodies for 1 hour. Proteins were visualized using the Pierce™ ECL Western blotting substrate (#32132X3; Thermo, Waltham, MA, USA) and analyzed using ImageJ software. β-actin was used as the internal control.

2.12 Transepithelial/Endothelial Resistance (TEER)

Caco-2 cells (5×10^4) were inoculated in the upper chamber of Transwell plates. After 2 days, Culture medium (200 µL) was added to the upper chamber and 1 mL to the lower chamber. After two days, TEER was measured using an EVOM cell potentiometer (#EVM-MT-03-01; World Precision Instruments Inc., Sarasota, FL, USA). Measurements were taken in triplicate, and average resistance values (Ω) were multiplied by the effective surface area to calculate TEER.

2.13 Statistical Analysis

Data are expressed as mean $\pm$ standard error of the mean (SEM). Statistical differences were analyzed using one-way or two-way analysis of variance (ANOVA) followed by Tukey's post-hoc test in GraphPad Prism (version 8.0; GraphPad Software, San Diego, CA, USA). A p-value of <0.05 was considered statistically significant.

3. Results

3.1 Dex Ameliorates Cerebral Infarction and Brain Injury Induced by MCAO

Dex administration significantly reduced cerebral infarction volume in the MCAO model, as shown in Fig. 1A,B, with the infarcted area reduced by ~40% compared to controls ($p < 0.01$). Additionally, Dex improved neurological scores (Fig. 1C) and decreased neuronal apoptosis in the cortical region (Fig. 1D,E), as evidenced by reduced TUNEL-positive neurons. Transcript levels of pro-inflammatory cytokines tumor necrosis factor (TNF)-α, IL-6, and IL-1β were significantly lowered by Dex treatment (Fig. 1F–H). These findings collectively demonstrate that Dex mitigates ischemic brain injury induced by reducing infarction size, neuronal apoptosis, and inflammation.

3.2 Dex Protects against Intestinal Barrier Dysfunction in MCAO Models

Dex demonstrated a protective effect on intestinal barrier permeability in MCAO models. HE staining showed

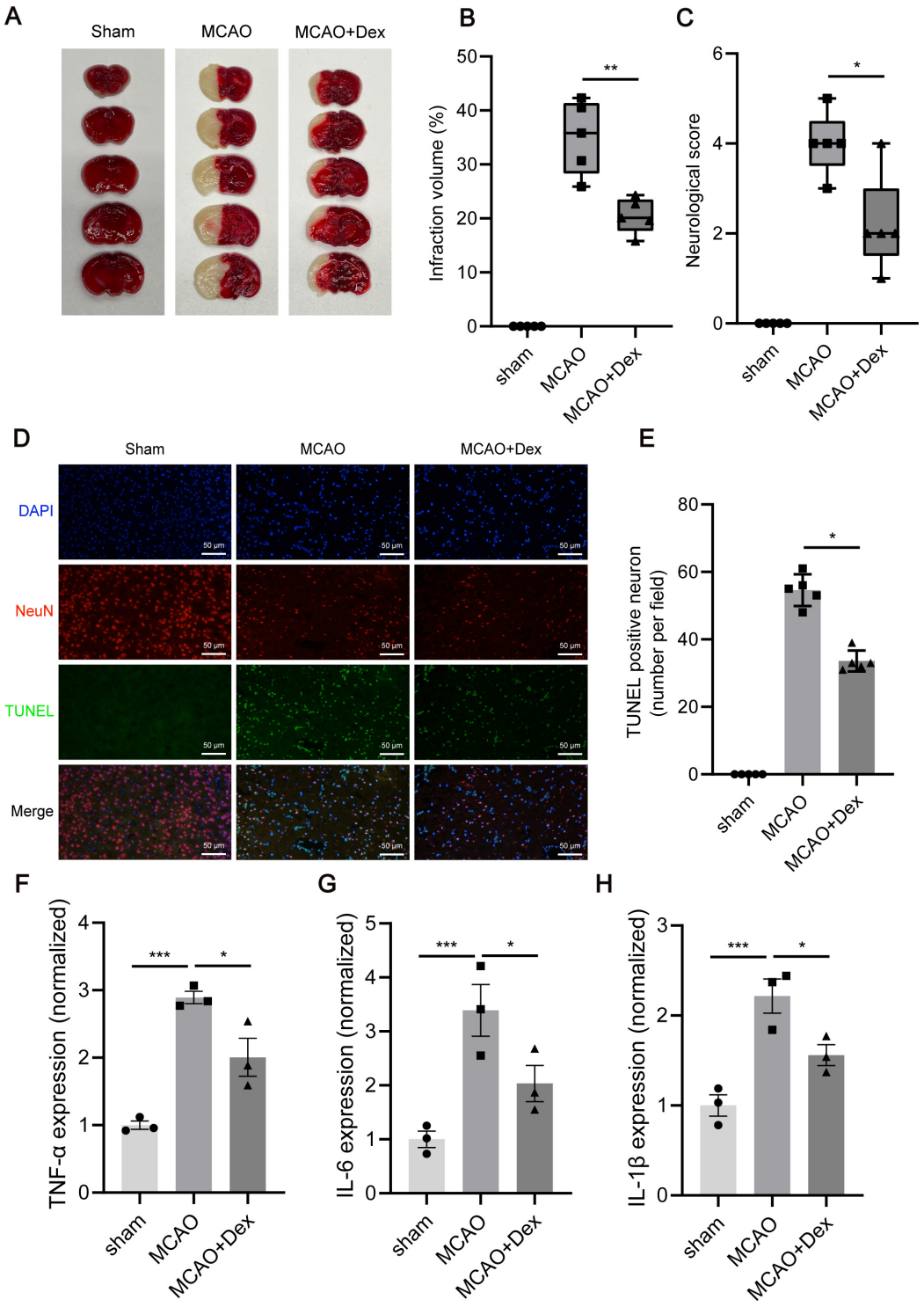

Fig. 1. Dex alleviated MCAO-induced brain injury in mouse models. (A,B) Representative TTC-stained brain sections and quantification of infarct volumes at 24 hours post-reperfusion. (C) Neurological deficit scores were evaluated using the Bederson scale at 24 hours post-reperfusion. (D,E) Double immunofluorescence staining of brain sections showing TUNEL-positive apoptotic neurons (TUNEL, green) co-labeled with NeuN (neuronal marker, red) and DAPI (nuclear stain, blue). The scale bar = 50 µm. Quantification of TUNEL-positive neurons is shown. (F–H) RT-qPCR analysis of pro-inflammatory cytokines TNF-α, IL-6, and IL-1β transcript levels in cerebral tissues. Data are expressed as mean ± SEM (n = 5). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ (one-way ANOVA with Tukey's post hoc test). Dex, Dexmedetomidine; MCAO, middle cerebral artery occlusion; TTC, Triphenyl Tetrazolium Chloride; RT-qPCR, reverse transcription-quantitative PCR; TNF, tumor necrosis factor; IL, interleukin; TUNEL, terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling; NeuN, neuronal nuclei.

minimal morphological changes in intestinal villi, which were insignificant across groups (Fig. 2A,B). Dex significantly reduced intestinal permeability, as measured by FITC-dextran levels ($p < 0.05$, Fig. 2C) and endotoxin levels (Fig. 2D). Additionally, Dex partially restored the expression of tight junction proteins, including occludin, zonula occludens-1 (ZO-1), and epithelial cadherin (E-cadherin) (Fig. 2E,F), supporting the structural integrity of the intestinal barrier dysregulation. These findings suggest that Dex improves MCAO-induced intestinal barrier dysregulation through permeability reduction and tight junction protein recovery.

3.3 Dex Weakens LPS-induced Barrier Dysfunction in Caco-2 Monolayers

To further validate the effect of Dex on intestinal barrier integrity, we used LPS-stimulated Caco-2 monolayers to mimic increased intestinal permeability following MCAO. LPS significantly downregulated E-cadherin, occludin, and ZO-1 protein levels in monolayers. However, Dex treatment partially restored E-cadherin protein levels but did not successfully recover occludin and ZO-1 levels (Fig. 2G,H). Given the role of FAK in regulating intestinal barrier function through TJ redistribution in epithelial cells, we assessed FAK protein levels. LPS stimulation decreased FAK expression, alleviated by Dex treatment (Fig. 2G,H). Additionally, as measured by TEER assays, transmembrane resistance was significantly reduced by LPS stimulation but was remarkably improved following Dex treatment (Fig. 2I). These findings demonstrate that Dex alleviates LPS-induced barrier dysfunction in Caco-2 monolayers.

3.4 Dex Promotes Intestinal Permeability Repair via the α2AR/FAK Pathway

Building on the cell-based findings, subsequent experiments assessed whether Dex ameliorates MCAO-induced intestinal permeability alterations through the α2AR/FAK pathway. Antagonists α2AR (SKF 86466) or FAK (BI 853520) were used in MCAO mouse models. Neither antagonist significantly altered the intestinal morphology of Dex-treated mice (Fig. 3A,B). However, both SKF 86466 and BI 853520 markedly reversed Dex-mediated improvements in intestinal permeability, as evidenced by increased serum FITC-dextran fluorescence and endotoxin levels (Fig. 3C,D). Furthermore, while Dex treatment improved MCAO-induced reductions α2AR, FAK, and E-cadherin protein levels, SKF 86466 reversed α2AR and FAK alterations, and BI 853520 reversed FAK and E-cadherin alterations but not α2AR expression (Fig. 3E,F). These findings indicate that Dex promotes intestinal barrier repair via activation of the α2AR/FAK pathway.

3.5 Inhibition of the α2AR/FAK Pathway Impairs the Protective Effect of Dex on MCAO-induced Brain Injury

To assess whether α2AR/FAK pathway inhibition impacts Dex's neuroprotective effects, SKF 86466 or BI 853520 were administered in MCAO mouse models. Dex-mediated reductions in cerebral infarct size were partially reversed after either antagonist (Fig. 4A,B). Similarly, Dex-induced improvements in neurological function scores were diminished following antagonist treatment (Fig. 4C). These results confirm that Dex attenuates MCAO-induced brain injury by activating the α2AR/FAK pathway. Together, the results demonstrate that Dex repairs intestinal barrier dysregulation and ameliorates MCAO-induced brain injury by modulating this signaling pathway (Fig. 5).

4. Discussion

Patients with IS face not only neurological deficits but also non-neurological complications, such as gastrointestinal dysfunction, immunosuppression, and bacterial infection [39]. Research has highlighted that bacteria found in patients with IS' blood, urine, or sputum often originate from the gut [40]. IS-induced disruption of intestinal barrier function leads to bacterial translocation, immune activation, and inflammatory responses, exacerbating systemic complications [41]. Thus, managing intestinal barrier dysfunction after cerebral infarction is vital. This study demonstrated that Dex improves MCAO-induced brain injury by repairing intestinal barrier dysregulation, likely through the α2AR/FAK pathway. This offers a novel perspective on the relationship between intestinal function and brain injury.

The gastrointestinal tract serves to digest nutrients and shield the body from bacteria and toxins through its intact barrier [42]. When this barrier is compromised, increased permeability allows bacterial translocation and endotoxins to enter the bloodstream, triggering inflammation and systemic changes [43]. This is particularly detrimental in IS patients, as it worsens recovery by promoting inflammatory responses and hindering neurological restoration [44]. Elevated bacterial metabolites, such as lipopolysaccharides, polarize microglia into the pro-inflammatory M1 type, exacerbating the release of pro-inflammatory cytokines and cytotoxic substances, further impairing neurological function [45]. This evidence underscores the need to protect intestinal barrier integrity to improve IS outcomes.

The integrity of the intestinal barrier depends on adherence and tight junctions, which regulate the selective passage of molecules and maintain epithelial cohesion. Key proteins such as E-cadherin, occludin, and ZO-1 play pivotal roles in preserving this barrier [46]. In IS, intestinal barrier disruption leads to the infiltration of bacterial metabolites, provoking inflammatory responses mediated by immune cells such as $\gamma\delta$T cells. These cells secrete IL-17, which triggers downstream pathways like mitogen-activated protein kinases (MAPK) and nuclear factor-kappaB, contributing to immune cell recruitment and

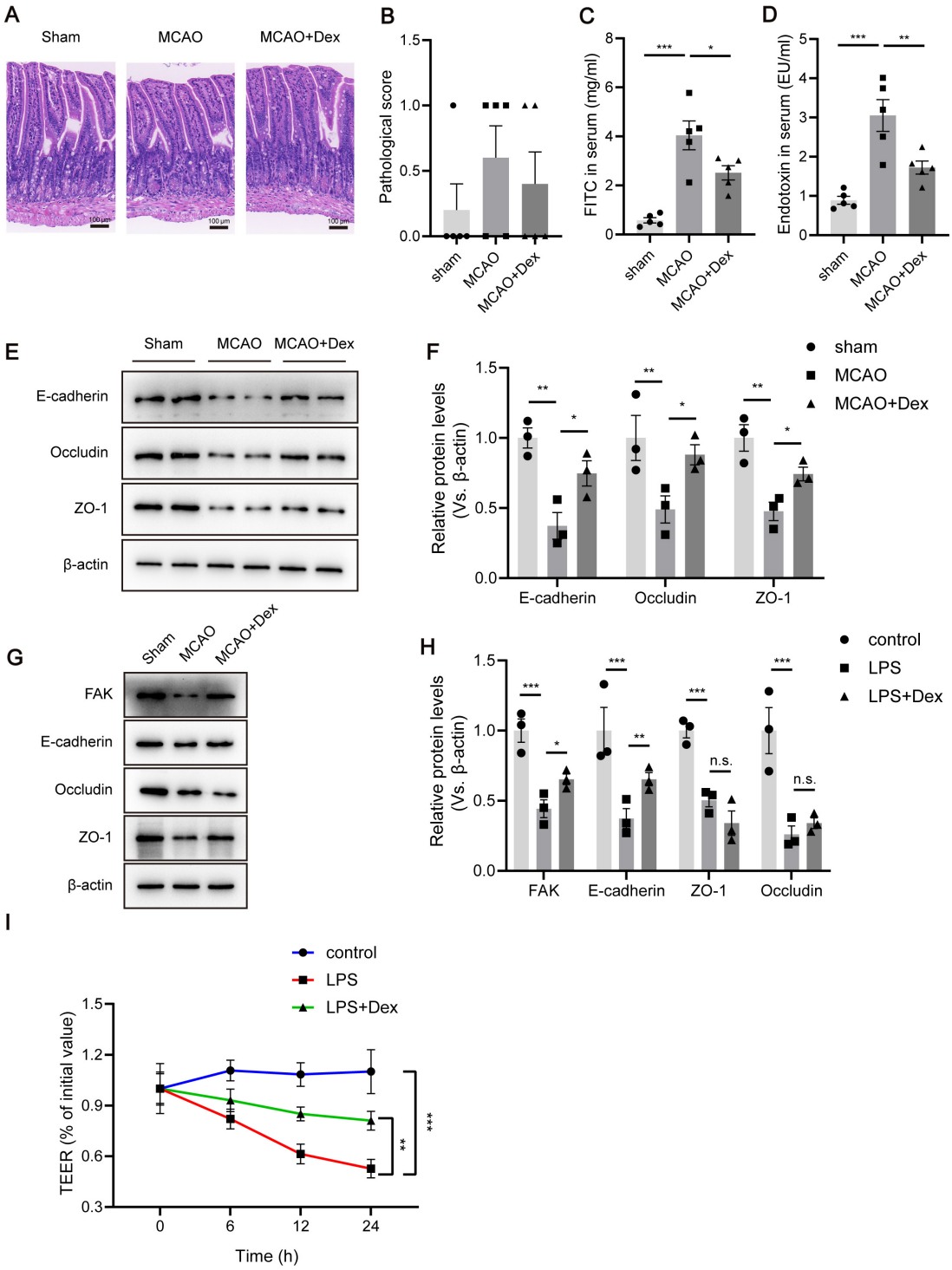

Fig. 2. Dex ameliorates intestinal barrier dysfunction in MCAO-induced mice and LPS-treated Caco-2 monolayers. (A,B) Representative HE-stained images of small intestinal tissue sections and their corresponding histological (n = 5). The scale bar = 100 μm. (C) Quantification of serum FITC-dextran levels to evaluate intestinal permeability (n = 5). (D) Serum endotoxin levels were measured using ELISA (n = 5). (E,F) Representative western blot images and quantification of TJ proteins (occludin, ZO-1) and adherens junction proteins (E-cadherin) in small intestinal tissues (n = 3). (G,H) Western blot analysis of FAK, E-cadherin, ZO-1, and occludin protein levels in Caco-2 monolayers under control, LPS, LPS+Dex (n = 3). (I) Transmembrane resistance of Caco-2 monolayers was measured using TEER assays (n = 3). Data are shown as mean ± SEM. Notes: rectangles, triangles, and circles represent samples in sham, MCAO, and MCAO+Dex, respectively. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ (one-way or two-way ANOVA with Tukey's post hoc test); n.s., no significant; LPS, lipopolysaccharide; HE, Hematoxylin-eosin; TEER, transepithelial/endothelial resistance; FAK, focal adhesion kinase; ZO-1, zonula occludens-1; E-cadherin, epithelial cadherin.

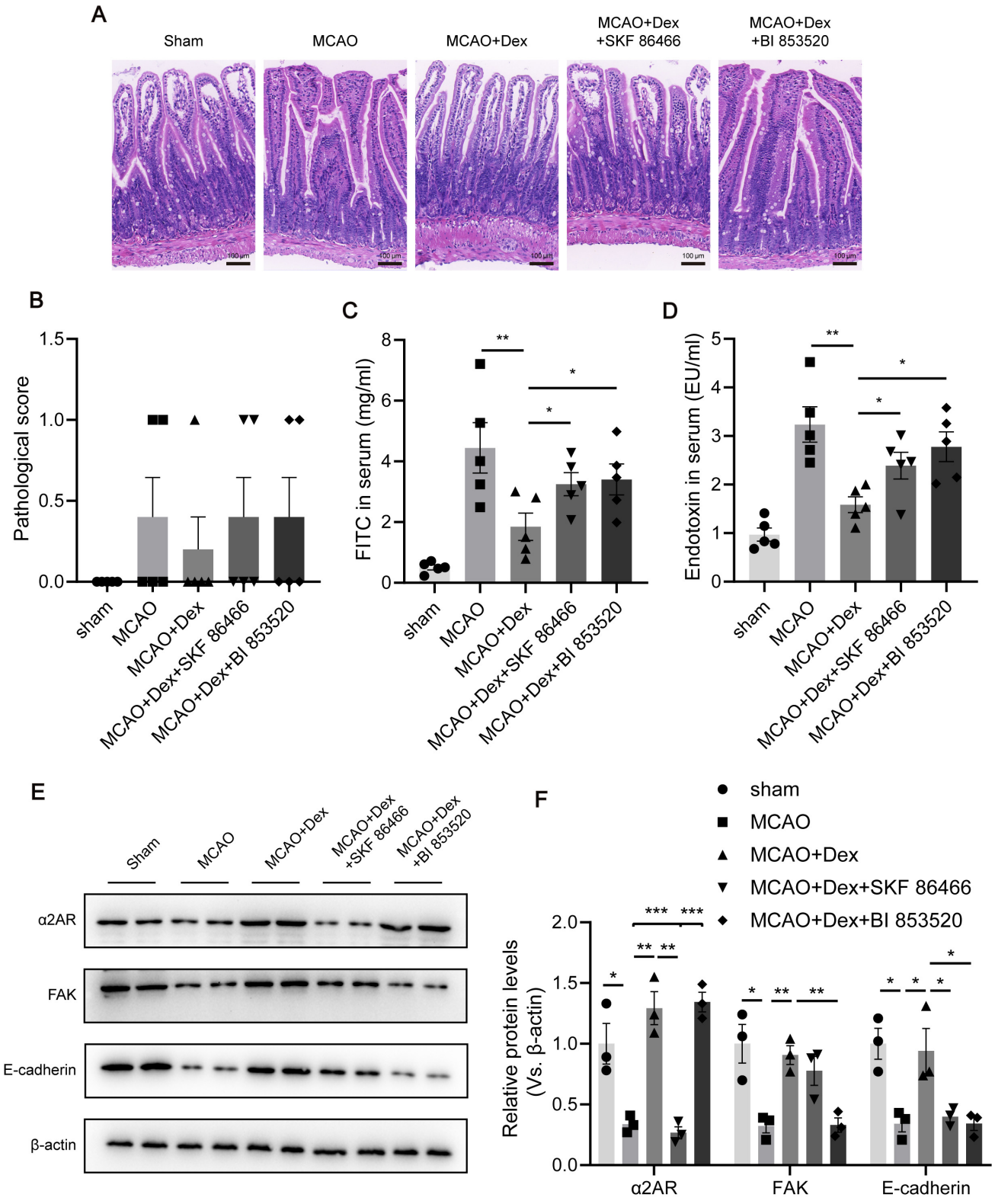

Fig. 3. Dex promotes intestinal permeability repair by the α2AR/FAK pathway in MCAO mice. (A,B) Representative H&E-stained images and histological scoring of small intestinal tissues from different groups (n = 5). The scale bar = 100 µm. (C,D) Serum FITC-dextran levels and endotoxin concentrations were measured to assess intestinal permeability in different groups (n = 5). (E,F) Western blot analysis and quantification of α2AR, FAK, and epithelial cadherin (E-cadherin) protein expression in small intestinal tissues (n = 3). Data are presented as the mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ (one-way ANOVA with Tukey's post hoc test). α2AR, α2-adrenoceptor; FITC, fluorescein isothiocyanate.

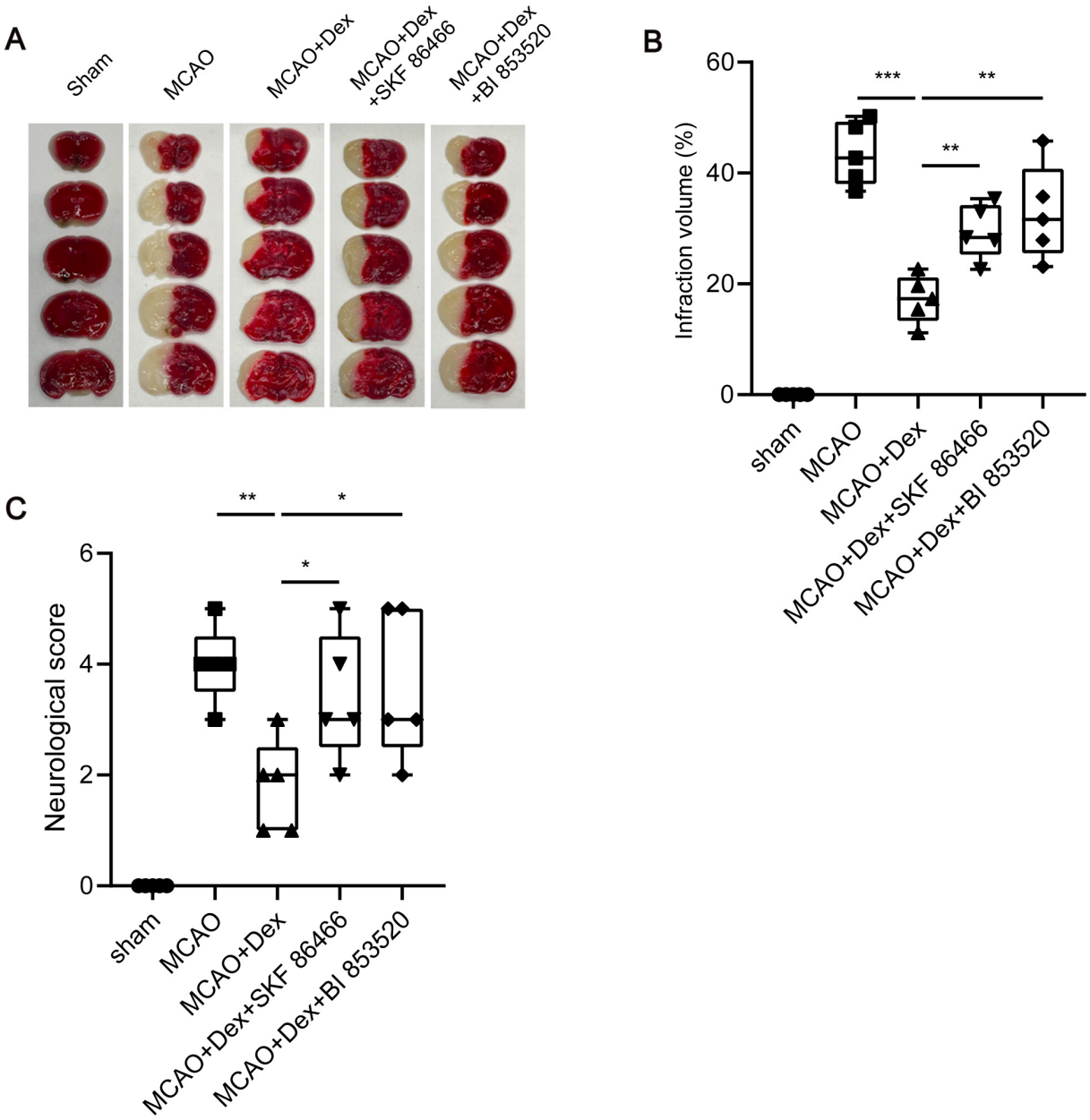

Fig. 4. Dex alleviates brain injury following MCAO via the α2AR/FAK signaling pathway. (A,B) Representative TTC-stained images and quantification of infarct volumes in mice across different groups (n = 5). (C) Neurological deficit scores were evaluated using the Bederson score in mice from different groups (n = 5). Data are presented as the mean ± SEM. Notes: rectangles, triangles, and circles represent samples in sham, MCAO, and MCAO+Dex, respectively. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ (one-way ANOVA with Tukey's post hoc test).

inflammatory infiltration [47–49]. Studies have shown that IL-17 binds to its receptor, activating pathways promoting neutrophil recruitment, dendritic cell activation, and tissue infiltration, exacerbating inflammation and tissue damage [50–52]. Modulating intestinal barrier integrity and controlling immune-inflammatory pathways may offer a therapeutic target for improving IS recovery.

Animal models of cerebral infarction are primarily constructed using rodents because they are easy to manage, have a short growth cycle, and incur low feeding costs [53]. In this study, MCAO-induced mice were employed to investigate the effects of Dex on cerebral infarction-induced brain injury and intestinal barrier dysfunction. The results demonstrated that Dex reduced infarction volume, improved neurological scores, decreased neuronal apoptosis, and mitigated inflammatory responses in MCAO-induced mice. These effects were accompanied by decreased FITC-dextran and endotoxin levels in serum and increased protein expression of E-cadherin, occludin, and ZO-1 in small intestinal tissues. Moreover, Dex treatment restored E-

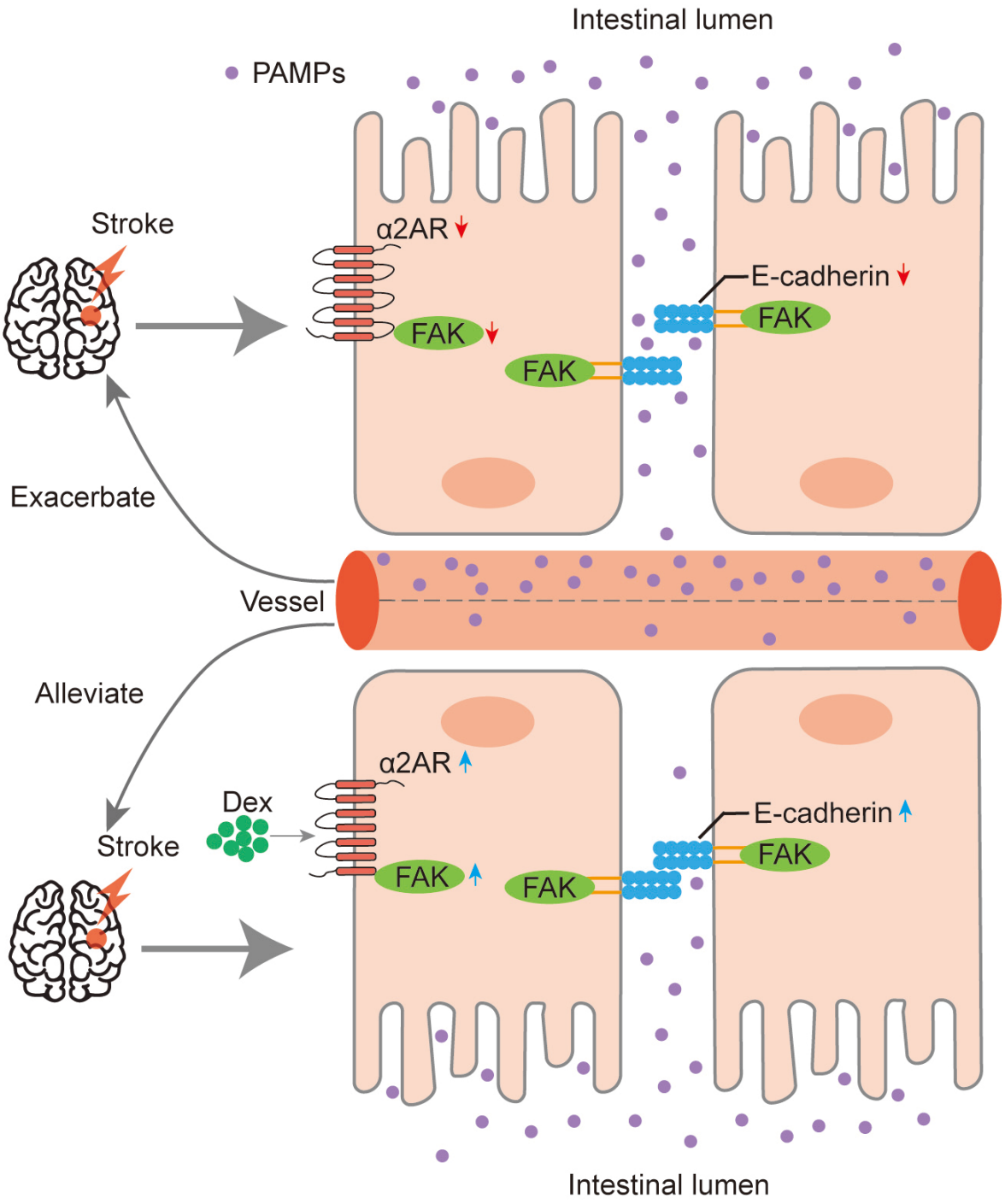

Fig. 5. Schematic illustration of Dex ameliorating stroke via the α2AR/FAK pathway-mediated intestinal barrier repair. The diagram demonstrates how Dex treatment reduces MCAO-induced brain injury by activating the α2AR/FAK signaling pathway and fostering intestinal barrier integrity. This pathway alleviates systemic inflammation and protects against secondary brain damage (A chart was created using adobe illustrator). PAMPs, pathogen-associated molecular patterns.

cadherin, occludin, and ZO-1 in small intestinal tissues. Moreover, Dex treatment restored TEER value and partially recovered tight junction protein levels in LPS-induced Caco-2 monolayers. These findings suggest that Dex alleviated cerebral infarction-induced brain injury by enhancing intestinal barrier integrity.

α2AR, a subfamily of adrenergic receptors, couples with and activates G proteins to trigger intracellular signaling cascades [54]. It has three isoforms—α2AR-A, α2AR-B, and α2AR-C—distributed widely in the central nervous system and peripheral tissues [55]. In the intestine, α2AR regulates Cl^- secretion in colonic

epithelial cells [56] and promotes epithelial cell proliferation via MAPK/extracellular signal-regulated kinase pathways [57]. Activation of α2AR by Dex initiates downstream pathways that mediate various biological effects [58–60], including modulation of vascular injury-induced pulmonary microvascular permeability through the α2AR/FAK pathway [61]. Additionally, FAK has been shown to improve intestinal epithelial integrity and reduce permeability in other models [62,63]. Our study examined whether Dex exerts its protective effects via the α2AR/FAK pathway. Dex impaired LPS-induced downregulation of FAK in Caco-2 monolayers, while α2AR and FAK inhibitors reversed Dex's effects on serum FITC-dextran levels, endotoxin concentrations, and E-cadherin expression in MCAO mice. Furthermore, these inhibitors negated Dex's neuroprotective effects, as evidenced by increased infarct volumes and worsened neurological scores. These findings suggest that Dex ameliorates IS-induced brain injury by enhancing intestinal barrier integrity via the α2AR/FAK pathway.

Sex differences in Dex efficacy have been observed in a clinical study with varying responses reported in male and female patients [64]. However, this study exclusively used male mice, and the potential influence of gender on Dex's protective effects warrants further investigation. Additionally, while this study implicates the α2AR/FAK pathway in Dex's efficacy, the direct relationship between these proteins remains to be elucidated. Further experiments are needed to validate this interaction and to evaluate the long-term outcomes of Dex treatment on IS recovery.

5. Conclusions

In conclusion, the "gut bacteria-intestinal-brain axis" describes the bidirectional relationship between the brain and intestinal tract. Increased intestinal permeability, due to impaired barrier function, promotes bacterial translocation and toxin entry into the bloodstream, aggravating systemic inflammation and exacerbating IS outcomes. This study demonstrated that Dex alleviates MCAO-induced brain injury by repairing intestinal barrier dysfunction via the α2AR/FAK pathway. These findings provide novel insights into the link between intestinal function and brain injury and suggest that targeting the gut-brain axis may offer new strategies for mitigating secondary injury in IS patients.

Availability of Data and Materials

The datasets used and/or analyzed in this study are available from the corresponding author upon rational request.

Author Contributions

ZHL designed and performed the research. XTZ and YY conducted experiments. ZXS analyzed the data. 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 approval for animal experimental research (approval code: 2022111229001) for this study was granted by the Medical Ethics Committee of the First Affiliated Hospital of University of South China. Animal handling procedures were in complete compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

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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/FBL27159.

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