

Berberine Attenuates Neonatal Sepsis in Rats By Inhibiting FOXA1 and NF- κ B Signaling Transduction Via The Induction of miR-132-3p

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Research Article

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Abstract

Neonatal sepsis (NS) is a severe syndrome in newborns induced by infections, the initiation and development of which are closely related to the function of miRs. In the current study, the anti-NS effects of berberine, a functional component in Traditional Chinese medicine (TCM), were assessed by focusing on its interaction with miR-132-3p-mediated signaling. NS model was induced using cecal slurry (CS) and handled with berberine. The changes in survival rate, intestinal structure, systemic inflammation, miR-132-3p level, and activities of FOXA1 and NF- κ B pathways were detected. The data showed that berberine increased survival rate of NS mice. The intestinal injuries induced by CS were also attenuated by berberine, which was associated with the inhibited production of systemic IL-6, IL-1 β , and TNF- α . At molecular level, the expression of miR-132-3p was up-regulated, suppressing the expressions of FOXA1, p-I κ B α , and p65, while inducing the expression of I κ B α . The effects of berberine on NS-induced impairments were blocked by the injection of miR-132-3p antagomir, which reduced survival rate, exacerbated intestinal injuries, induced systemic inflammation, and re-activated FOXA1 and NF- κ B pathways. Collectively, the findings outlined in the current study indicated that berberine had solid protective effects on newborn mice against NS-induced symptoms, and the effects depended on the up-regulation of miR-132-3p.

Introduction

Sepsis is defined as a complex syndrome initiated by the response of host body to multiple types of infection. The disorder results in systemic inflammation and severe organ dysfunction [1], and remains a major causable factor of morbidity and mortality of world population [2]. It is well recognized that the highest incidence of sepsis is observed in elderly people. However, investigations in the last decade demonstrate that infants are also vulnerable to sepsis. The study by Brocklehurst showed that four of every 10 neonates with sepsis died or experienced major disability even after timely treatments [3]. The incidence of neonatal sepsis (NS) is estimated to be around 25%~30%, but the mortality rate can be as high as 52% [4]. Although plenty efforts have been made to improve the clinical prognosis of NS patients, the management of the disorder stays a great challenge in clinic. Thus, the exploration of effective medicines for the treatment of NS needs prompt solution.

The emerging studies regarding NS have substantially improved the understanding of the pathology of the disease and revealed the diagnostic and treatment potentials of different biological factors in the progression of NS. Of which, microRNAs (miRs) have elicited lots of interest of scientists and clinicians for their involvement in sepsis [5-9]. MiRs are a class of endogenous and non-coding short RNA molecules (~22 nt). Currently, more than 2600 types of miRs have been identified and are involved in the regulation of more than 30% genes in human cells. Regarding the roles of miRs in the development of sepsis, the study by Li et al. indicated that miR-25 was involved in the treatment of ginkgolide A against sepsis [10] and the study by Zhang et al. showed that miR-22-3p could serve as an early diagnostic biomarker for sepsis in clinic [11]. Moreover, miRs such as miR-146a, miR-574-5p, miR-122, and miR-133a have been reported to have reference value for the clinical diagnosis and prognosis of sepsis [5-9].

However, even with the evident interaction between sepsis and miRs, few studies have paid attention to the involvement of miRs in the development and treatment of NS. Given the multiple functions of miRs in sepsis, it is reasonable to further explore the roles of miRs in NS. In the study by Benet et al., the authors demonstrated that miR-132-3p were down-regulated in septic newborns [9]. Additionally, one of the downstream effectors of the miR (http://www.targetscan.org/cgi-bin/targetscan/vert_71/view_gene.cgi?rs=ENST00000250448.2&taxid=9606&members=miR-132-3p/212-3p&showcnc=0&shownc=0&subset=1), FOXA1 is reported to be a promoter for septic injuries [12]. Thus, the target regulation of miR-132-3p/FOXA1 axis may be a promising strategy for the effective diagnosis and management of NS.

Berberine is an isoquinoline alkaloid isolated from *Coptis chinensis*, which have been widely used in different Traditional Chinese Medicine (TCM) formula for centuries. Previous study showed that berberine could increase the survival rate of sepsis mice by affecting the activity of HMGB1 [13]. Berberine also inhibited the activation and expression of transcription factor in sepsis by downregulating NF- κ B pathway [14]. The studies collectively confirmed the anti-sepsis effects of the compound. Moreover, the study by Ge et al. showed that berberine could influence the level of miR-132-3p [15]. Although the study was performed in neural cells, it might represent the potential interaction between berberine and miR-132-3p. Thus, in the current study, we hypothesized that berberine could attenuate NS by modulating miR-132-3p/FOXA1 axis. To verify the hypothesis, NS was induced in newborn rats using cecal slurry (CS) model and handled with berberine via intraperitoneal injection. The influence of berberine on systemic inflammation, bowel tissue structure, and miR-132-3p/FOXA1 axis was investigated.

Materials And Methods

Animals preparation and NS model induction

All the animal experiments were performed under the approval of the Animal Care and Use Committee of Maternal and Child Health Care Hospital of Zibo, and were carried out in accordance with the Animal Welfare Law and the guidelines for animal care and use of the National Institutes of Health. Neonatal mice (5–7 days old) bred by the C57BL/6J mice were prepared by Wanleibio (Shenyang, China) and maintained with free access to standard care, diet, and drinking. In the current study, the cecal slurry (CS) model was established to induce NS following the of method by Wynn et al. [16] with modifications: briefly, the adult mice were sacrificed and the cecal contents were collected, and mixed with 5% glucose solution to produce CS solution (80 mg/ml). Then 60 neonatal mice were randomly divided into four groups (15 for each group): Sham group, mice received intraperitoneal injection of 0.9% saline. CS group, mice received intraperitoneal injection of CS at a concentration of 1.3 mg/g body weight according to the study by Li et al. [17]; CS + Low group, mice received intraperitoneal injection of CS and low dose of berberine (50 μ g); CS + High group, mice received intraperitoneal injection of CS and high dose of berberine (100 μ g). 24 h after the injection, the survival rate of the mice was calculated in a 72-h period and the left mice were sacrificed using overdose (150 mg/kg BW) pentobarbital sodium. The small intestine and whole blood samples were collected from the survival mice for the subsequent assays.

Hematoxylin-eosin (H&E) staining and intestinal injury score

Intestinal injuries were detected with H&E staining: the tissues were immersed, fixed in 8% formalin for embedding, and sectioned. Then the sections were incubated with hematoxylin and eosin. The histological changes in the tissues were detected under a microscope under 200× magnification and the injury degree of the tissues were evaluated by two investigators blind to the experimental designment following previous methods [18]. Briefly, a score of 0 represented normal mucosa; a score of 1 represented the development of subepithelial Gruenhagen's space, vacuolization or subepithelial lifting limited to the lamina propria or tips of villi; a score of 2 represented epithelial lifting and vacuolization greater than half of the villi, villi distortion, or mucosal ulceration and disintegration of the lamina propria.

Enzyme linked sorbent immune assay (ELISA)

The blood levels of cytokines IL-6 (SEA079Hu), IL-1 β (SEA563Hu), and TNF- α (SEA133Hu) were detected using corresponding kits purchased from USCN Business Co., Ltd (China).

Reverse transcription quantitative PCR (RT-qPCR)

Total RNA in small intestine tissues was collected and extracted using a RNA extraction kit (QIGEN, Duesseldorf, Germany), and was reversely transcribed into cDNA (50 ng/ μ l) according to the instructions of PrimeScriptTM RT reagent Kit (Beijing, China). The expression of miR-132-3p (forward: 5'-GCGCGTAACAGTCTACAGCCA-3'; reverse: 5'-AGTGCAGGGTCCGAGGTATT-3') was detected using a Real-time PCR Detection System following routine protocol (ABI 7900, Shanghai, China) and the relative expression level of miR-132-3p was calculated using the formula of $2^{-\Delta\Delta Cq}$ in reference to Sham group (normalized as 1).

Western blotting assay

Total protein products of small intestinal tissues were extracted using the RIPA lysis buffer and were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Then the membranes were incubated with primary antibodies against different antibodies (Table S1). After washing, protein blots were developed using Beyo ECL Plus reagent. The results were scanned in the Gel Imaging System (WD-9413B, Liuyi Factory, China) and the integrated optical densities were recorded. The relative expression levels of the different proteins were calculated with Gel-Pro-Analyzer (Media Cybernetics, USA).

Inhibition of miR-132-3p in mice

To verify the interaction between miR-132-3p and berberine in the treatment of NS, the level of miR-132-3p was inhibited using specific antagomir 24 h prior to CS and berberine administrations. Then the changes in survival rate, small intestine histology, systemic inflammation, and activities of FOXA1 and NF- κ B pathways were detected as described above.

Statistical analysis

All the continuous data were represented as mean \pm standard deviation (SD). The difference was calculated using one-way analysis of variance (ANOVA) followed by multiple comparisons with Tukey method. Survival was compared using log-rank test. All the statistical analyses and graphic manipulation were conducted using Graphpad Prism version 6.0 (GraphPad Software, Inc., San Diego, CA) with a significant level of 0.05 (two-tailed *P* value).

Results

Berberine improved the survival rate of NS mice

To confirm the lethal effect of NS complications on mice, we calculated the survival rate of model animals after NS induction. As shown in Figure 1, the survival of NS mice was dramatically exacerbated in comparison to normal mice (40.0% vs. 93.3%, $p < 0.05$), indicating that the attack of NS was extremely negative to the survival of newborn mice. For NS mice co-administrated with CS and berberine, the survival rates obviously increased (Figure 1) (40% vs. 53.3% and 80%). However, of the two administrating doses, only the dose of 100 μ g led to a survival rate significantly higher than that of CS group ($p < 0.05$). Even for the mice administrated with high dose of berberine, the survival rate was significantly lower than that of Sham group ($p < 0.05$), which indicated that NS was a critical risk factor for the mortality of newborns. Collectively, although berberine could not entirely eliminate the negative effects induced by NS attack on the survival rate, the compound did protect mice against the mortality induced by the disorder.

Berberine attenuated intestinal injuries induced by NS in mice

Generally, the attack of sepsis will always induce injuries in the small bowel [19]. Thus, we detected the histological changes in small bowel using H&E staining and scored the injuries. In contrary to the intestinal architecture of healthy mice, the tissues of NS mice showed gross evidence of injury as well as evidence of progressive separation of the villi from the thickened basement membrane (Figure 2A). For mice administrated with berberine of both doses, the deterioration of intestine was obviously improved, further confirming anti-NS function of the compound. The injuries in small intestine were scored by two investigators. The results were in consistence with the detections of H&E: the injury score of CS group was much higher than that Sham group, but was reduced by berberine of both doses (Figure 2B) ($p < 0.05$).

Berberine suppressed the systemic inflammatory response induced by NS in mice

The induction of NS in mice increased the production of serum levels of IL-6, IL-1 β , and TNF- α (Figure 2C-2D) when compared with Sham group, confirming the initiation of inflammatory response associated with sepsis. The administrations of berberine suppressed the levels of the three cytokines, but no

significant difference was detected between the two doses regarding the effects on the production of cytokines.

Berberine up-regulated the expression of miR-132-3p while inhibited the activity of FOXA1 and NF- κ B pathways

To explain the potential mechanism driving the anti-NS function of berberine, the current study focused on the interaction between berberine and miR-132-3p as well as the downstream effectors. The induction of NS inhibited expression of miR-132-3p, while increased the expression of FOXA1 and activated NF- κ B pathway by suppressing the expression I κ B α and inducing the expressions of p-I κ B α and nuclear p65 (Figure 3). The data indicated that miR-132-3p might play an anti-NS role by influencing the activities of FOXA1 and NF- κ B pathways. For NS mice administrated with berberine, the expression level of miR-132-3p was restored and the activities of FOXA1 and NF- κ B pathways were blocked (Figure 3).

Anti-NS function of berberine depended in the up-regulation of miR-132-3p

The above data had indicated that the effects of berberine were associated with the function of miR-132-3p-mediated signaling transduction. However, whether the role of miR-132-3p in the anti-NS effects of berberine was indispensable needed further verification. Thus, the mice were injected with miR-132-3p antagomir 24 h prior to CS and berberine administrations to suppress the level of miR (Figure 4A). As shown in Figure 4B and Figure 4C, the injection of antagomir counteracted the effects of berberine on intestinal architecture: the tissues injury was deteriorated and the injury score was increased when compared with NS mice solely administrated with berberine. Moreover, the systemic levels of IL-6, IL-1 β , and TNF- α in Antagomir + berberine group were also significantly higher than those in NC + berberine group (Figure 4D-4F) ($p < 0.05$). At molecular level, the injection of miR-132-3p antagomir induced the activation of FOXA1 and NF- κ B pathways (Figure 5). However, compared with the effects of miR-132-3p antagomir on intestinal structure and systemic inflammatory, the current study didn't record significant influence on the survival rate of NS mice by antagomir (Figure S1).

Discussion

NS is one of the most severe factors causing neonatal morbidities. Worse still, due to the immaturity of newborn's immune system, the mechanism underlying NS is far more complicated than those occur in adults [20]. The current knowledge regarding the pathogenesis of NS demonstrates that the uncontrolled activation of inflammatory cascades due to the immature newborn immune system contributes to the initiation of catastrophic injuries in neonatal organs during the progression of sepsis. Thus, the understanding of molecular mechanism driving the onset of NS is paramount for the development of effective therapies against the disease in clinic. With emerging attention paid to the diagnosis and treatment of NS, different biomarkers with therapeutic potentials have been discovered. Of which, the role of miRs in the initiation and development of NS has become an attractive subject due to the diverse functions of miRs in different biological processes.

According to the study by Benet et al., miR-132-3p were down-regulated in septic newborns [9], indicating that the miR might play a protective role against the attack of NS. Thus, the current study further verified the possibility by inducing septic syndromes in newborn mice using CS method [16]. The data showed that the administration of CS decreased survival rate, induced small intestinal injuries, and initiated systemic inflammation in newborn mice, affirming the establishment of NS model. Moreover, the development of NS syndromes was associated with the down-regulation of miR-132-3p level, which was consistent with the report by Benet et al. [9]. Generally, miRs regulate the function of target gene by directing binding to the 3'UTR sequence. Based on the prediction of TargetScan, it was found that FOXA1 was a typical downstream effector of miR-132-3p. FOXA1 is an important member of FOXA family and is widely involved in cell development, proliferation, and apoptosis [12]. Moreover, the expression of FOXA1 is positively correlated with the onset of sepsis and considered as a pro-apoptosis factor in sepsis-induced organ failures [12]. Except for the direct regulation of the function of FOXA1, miR-132-3p could also exert anti-inflammatory effects by blocking NF- κ B signaling pathway [21]. The down-regulation of miR-132-3p during the development of NS will contribute to the NS-related syndromes by both inducing the activities of FOXA1 and NF- κ B signaling. Thus, the restored level of miR-132-3p might represent a promising therapeutic strategy for handling NS.

Berberine is a principle bioactive component isolated from *Rhizoma coptidis* that has been widely used for hundreds of years in Traditional Chinese Medicine (TCM) for the treatment of cardiovascular diseases, type 2 diabetes, and neurodegeneration diseases [22-24]. Regarding its effects on sepsis, the previous studies showed that the compound could attenuate septic syndromes in different systems [25, 26]. The effects were verified in the current study: the administrations of berberine of two doses increased survival rate, improved small intestinal structure, and inhibited systemic inflammation in NS mice. Based on the study by Ge et al., berberine had an evident regulatory function on the level of miR-132-3p in newborns [15]. Thus, we also explored the interaction between berberine and miR-132-3p for explaining the mechanism driving the anti-NS function of the compound. The data showed that the administrations of berberine could increase the level of miR-132-3p in NS mice, which contributed to the inhibition of FOXA1 and NF- κ B pathway. Additionally, the pre-injection of miR-132-3p antagomir could weaken the anti-NS effects of berberine, indicating that the induced level of miR-132-3p was indispensable for the function of berberine, at least in its treatment of NS syndromes.

Collectively, the current study confirmed the therapeutic potentials of miR-132-3p in NS treatment and the anti-NS effects of berberine. Moreover, the findings also inferred that miR-132-3p played a critical role in the protective effects of berberine against septic syndromes in newborns by suppressing the activities of its downstream effectors, such as FOXA1 and NF- κ B signaling. Once the level of miR-132-3p was inhibited, the anti-NS effects of berberine was substantially counteracted. Based on the data in the current study, it is reasonable to assess the therapeutic potentials of more miRs in the anti-NS effects of natural compound such as berberine, which will provide more opportunities for improving the clinical outcomes of NS patients.

Declarations

Funding

Not applicable

Conflicts of interests

The authors declare that they have no competing interests

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

BHL collected and analyzed the data, and wrote the draft. SPN, HLG, and CKY collected the data. DW designed the experiment, revised the draft, and approved the submission.

Acknowledgments

Not applicable

Ethics approval

All the animal experiments were performed under the approval of the Animal Care and Use Committee of Maternal and Child Health Care Hospital of Zibo, and were carried out in accordance with the Animal Welfare Law and the guidelines for animal care and use of the National Institutes of Health.

References

1. Shane, A. L., and B. J. Stoll. 2014. Neonatal sepsis: progress towards improved outcomes. *J Infect* 68 Suppl 1:S24-32. doi:10.1016/j.jinf.2013.09.011.
2. Burnham, J. P., M. A. Lane, and M. H. Kollef. 2015. Impact of Sepsis Classification and Multidrug-Resistance Status on Outcome Among Patients Treated With Appropriate Therapy. *Crit Care Med* 43 (8):1580-1586. doi:10.1097/ccm.0000000000001013.
3. Esposito, E., D. Impellizzeri, E. Mazzone, G. Fakhfour, R. Rahimian, C. Travelli, G. C. Tron, A. A. Genazzani, and S. Cuzzocrea. 2012. The NAMPT inhibitor FK866 reverts the damage in spinal cord injury. *J Neuroinflammation* 9:66. doi:10.1186/1742-2094-9-66.
4. Brocklehurst, P., B. Farrell, A. King, E. Juszcak, B. Darlow, K. Haque, A. Salt, B. Stenson, and W. Tarnow-Mordi. 2011. Treatment of neonatal sepsis with intravenous immune globulin. *N Engl J Med* 365 (13):1201-1211. doi:10.1056/NEJMoa1100441.

5. Karam, R. A., H. E. Zidan, N. A. Karam, D. M. Abdel Rahman, and O. S. El-Seifi. 2019. Diagnostic and prognostic significance of serum miRNA-146-a expression in Egyptian children with sepsis in a pediatric intensive care unit. *J Gene Med* 21 (11):e3128. doi:10.1002/jgm.3128.
6. Wang, H., K. Meng, Wj Chen, D. Feng, Y. Jia, and L. Xie. 2012. Serum miR-574-5p: a prognostic predictor of sepsis patients. *Shock* 37 (3):263-267. doi:10.1097/SHK.0b013e318241baf8.
7. Leelahavanichkul, A., P. Somparn, T. Panich, W. Chanchaoenthana, J. Wongphom, T. Pisitkun, N. Hirankarn, and S. Eiam-Ong. 2015. Serum miRNA-122 in acute liver injury induced by kidney injury and sepsis in CD-1 mouse models. *Hepato Res* 45 (13):1341-1352. doi:10.1111/hepr.12501.
8. Tacke, F., C. Roderburg, F. Benz, D. V. Cardenas, M. Luedde, H. J. Hippe, N. Frey et al. . 2014. Levels of circulating miR-133a are elevated in sepsis and predict mortality in critically ill patients. *Crit Care Med* 42 (5):1096-1104. doi:10.1097/ccm.000000000000131.
9. Dhas, B. B., V. R. Dirisala, and B. V. Bhat. 2018. Expression Levels of Candidate Circulating microRNAs in Early-Onset Neonatal Sepsis Compared With Healthy Newborns. *Genomics Insights* 11:1178631018797079. doi:10.1177/1178631018797079.
10. Li, J., J. Chen, Y. Yang, R. Ding, M. Wang, and Z. Gu. 2021. Ginkgolide A attenuates sepsis-associated kidney damage via upregulating microRNA-25 with NADPH oxidase 4 as the target. *Int Immunopharmacol* 95:107514. doi:10.1016/j.intimp.2021.107514.
11. Zhang, H., L. Che, Y. Wang, H. Zhou, H. Gong, X. Man, and Q. Zhao. 2021. Deregulated microRNA-22-3p in patients with sepsis-induced acute kidney injury serves as a new biomarker to predict disease occurrence and 28-day survival outcomes. *Int Urol Nephrol*. doi:10.1007/s11255-021-02784-z.
12. Lu, S., H. Wu, J. Xu, Z. He, H. Li, and C. Ning. 2020. SIK1AT1/miR-96/FOXA1 axis regulates sepsis-induced kidney injury through induction of apoptosis. *Inflamm Res* 69 (7):645-656. doi:10.1007/s00011-020-01350-0.
13. Lee, D., J. Bae, Y. K. Kim, M. Gil, J. Y. Lee, C. S. Park, and K. J. Lee. 2013. Inhibitory effects of berberine on lipopolysaccharide-induced inducible nitric oxide synthase and the high-mobility group box 1 release in macrophages. *Biochem Biophys Res Commun* 431 (3):506-511. doi:10.1016/j.bbrc.2012.12.143.
14. Gao, M. Y., L. Chen, L. Yang, X. Yu, J. P. Kou, and B. Y. Yu. 2014. Berberine inhibits LPS-induced TF procoagulant activity and expression through NF- κ B/p65, Akt and MAPK pathway in THP-1 cells. *Pharmacol Rep* 66 (3):480-484. doi:10.1016/j.pharep.2013.12.004.
15. Ge, Y., X. Song, J. Liu, C. Liu, and C. Xu. 2020. The Combined Therapy of Berberine Treatment with lncRNA BACE1-AS Depletion Attenuates A β (25-35) Induced Neuronal Injury Through Regulating the Expression of miR-132-3p in Neuronal Cells. *Neurochem Res* 45 (4):741-751. doi:10.1007/s11064-019-02947-6.
16. Wynn, J. L., P. O. Scumpia, M. J. Delano, K. A. O'Malley, R. Ungaro, A. Abouhamze, and L. L. Moldawer. 2007. Increased mortality and altered immunity in neonatal sepsis produced by generalized peritonitis. *Shock* 28 (6):675-683. doi:10.1097/SHK.0b013e3180556d09.

17. Li, Y., J. Ke, C. Peng, F. Wu, and Y. Song. 2018. microRNA-300/NAMPT regulates inflammatory responses through activation of AMPK/mTOR signaling pathway in neonatal sepsis. *Biomed Pharmacother* 108:271-279. doi:10.1016/j.biopha.2018.08.064.
18. Feinman, R., E. A. Deitch, A. C. Watkins, B. Abungu, I. Colorado, K. B. Kannan, S. U. Sheth et al. . 2010. HIF-1 mediates pathogenic inflammatory responses to intestinal ischemia-reperfusion injury. *Am J Physiol Gastrointest Liver Physiol* 299 (4):G833-843. doi:10.1152/ajpgi.00065.2010.
19. Shrum, B., R. V. Anantha, S. X. Xu, M. Donnelly, S. M. Haeryfar, J. K. McCormick, and T. Mele. 2014. A robust scoring system to evaluate sepsis severity in an animal model. *BMC Res Notes* 7:233. doi:10.1186/1756-0500-7-233.
20. Wynn, J. L., C. S. Wilson, J. Hawiger, P. O. Scumpia, A. F. Marshall, J. H. Liu, I. Zharkikh et al. . 2016. Targeting IL-17A attenuates neonatal sepsis mortality induced by IL-18. *Proc Natl Acad Sci U S A* 113 (19):E2627-2635. doi:10.1073/pnas.1515793113.
21. Fang, H., H. F. Li, Q. Pan, H. L. Jin, M. Yang, R. R. Wang, Q. Y. Wang, and J. P. Zhang. 2021. MiR-132-3p Modulates MEKK3-Dependent NF- κ B and p38/JNK Signaling Pathways to Alleviate Spinal Cord Ischemia-Reperfusion Injury by Hindering M1 Polarization of Macrophages. *Front Cell Dev Biol* 9:570451. doi:10.3389/fcell.2021.570451.
22. Feng, X., A. Sureda, S. Jafari, Z. Memariani, D. Tewari, G. Annunziata, L. Barrea et al. . 2019. Berberine in Cardiovascular and Metabolic Diseases: From Mechanisms to Therapeutics. *Theranostics* 9 (7):1923-1951. doi:10.7150/thno.30787.
23. Affuso, F., V. Mercurio, V. Fazio, and S. Fazio. 2010. Cardiovascular and metabolic effects of Berberine. *World J Cardiol* 2 (4):71-77. doi:10.4330/wjc.v2.i4.71.
24. Ahmed, T., A. U. Gilani, M. Abdollahi, M. Daglia, S. F. Nabavi, and S. M. Nabavi. 2015. Berberine and neurodegeneration: A review of literature. *Pharmacol Rep* 67 (5):970-979. doi:10.1016/j.pharep.2015.03.002.
25. Wang, Y., P. Du, and D. Jiang. 2020. Berberine functions as a negative regulator in lipopolysaccharide-induced sepsis by suppressing NF- κ B and IL-6 mediated STAT3 activation. *Pathog Dis* 78 (7). doi:10.1093/femspd/ftaa047.
26. Chen, H., Q. Liu, X. Liu, and J. Jin. 2021. Berberine attenuates septic cardiomyopathy by inhibiting TLR4/NF- κ B signalling in rats. *Pharm Biol* 59 (1):121-128. doi:10.1080/13880209.2021.1877736.

Figures

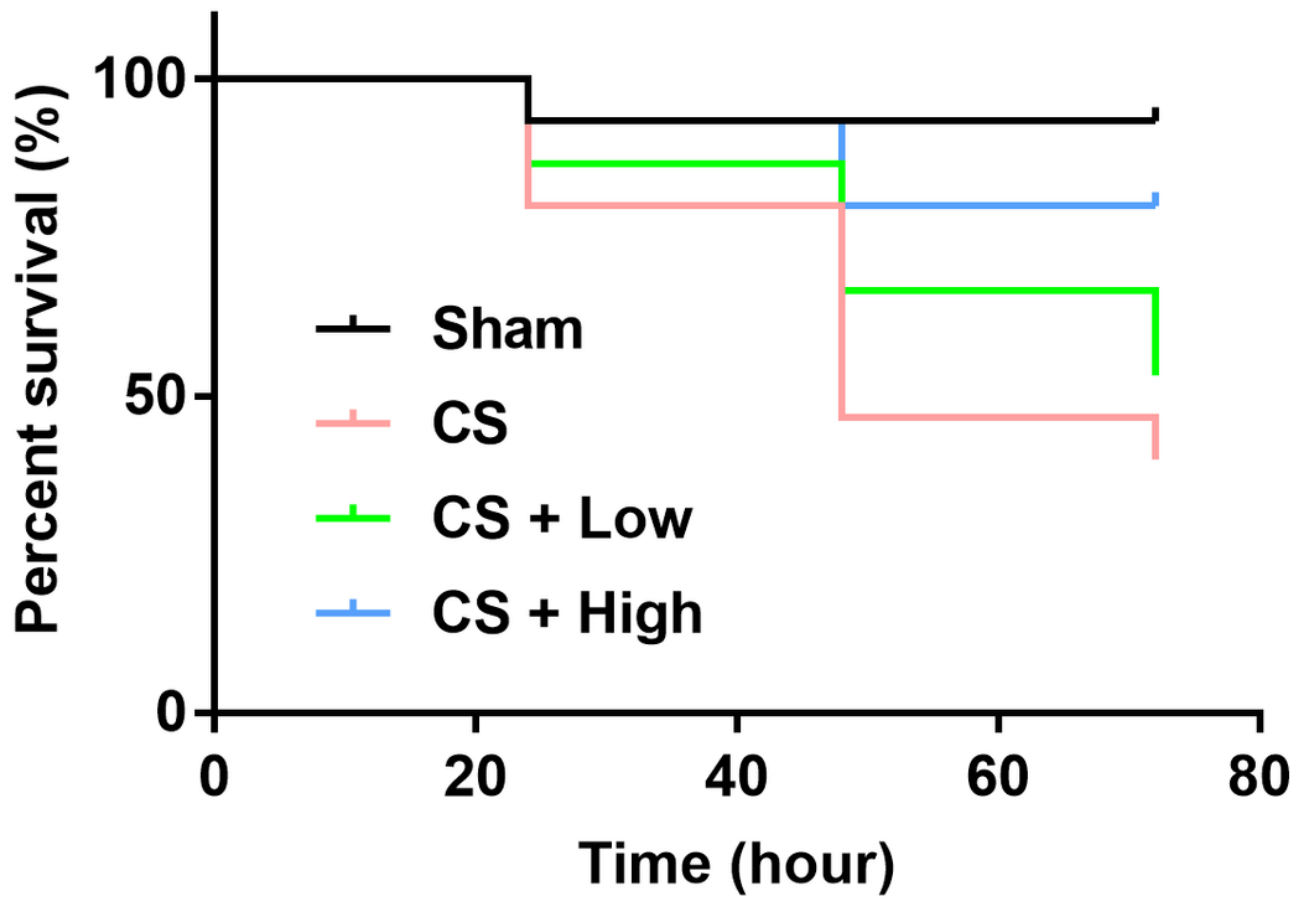


Figure 1

Effects of NS model and berberine administrations on the survival in mice. NS model was induced with 5-~7-day-old neonatal mice using cecal contents (CS) collected from adult mice. Berberine was administrated in doses along with CS. The survival rate of mice in different groups were calculated in a 72-h period after the administrations with log-rank test.

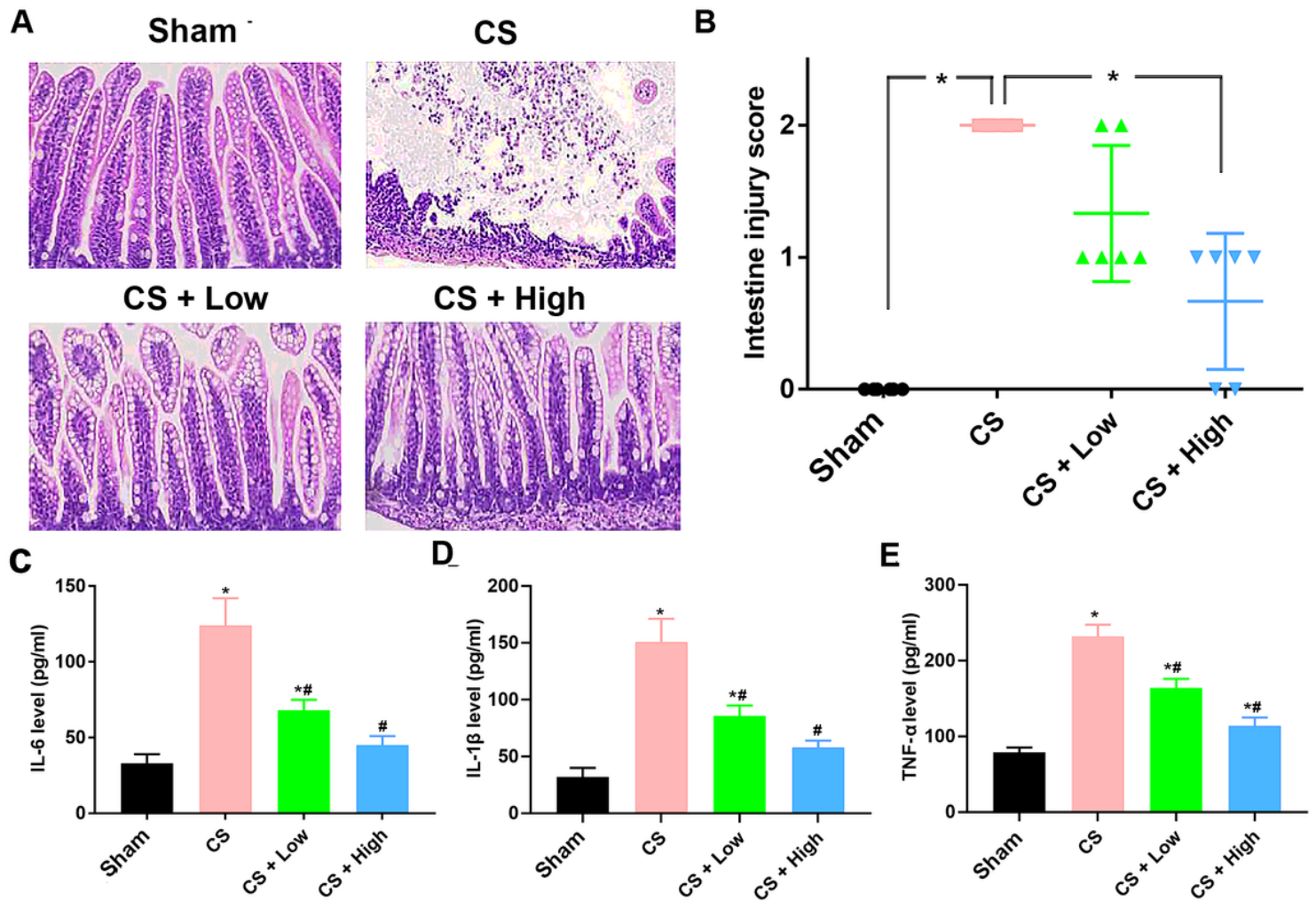


Figure 2

Effects of NS model and berberine administrations on the small intestinal structure and systemic inflammatory response in mice. The histological changes in small intestine of mice were detected using H&E staining and the injury degree was scored. The production of different cytokines was detected using corresponding kits. A) H&E staining detection of small intestinal structure. B) Quantitative analysis results of injury score. C) Quantitative analysis results of IL-6 level. D) Quantitative analysis results of IL-1 β level. E) Quantitative analysis results of TNF- α level. “*” represents p < 0.05 vs. Sham group. “#” represents p < 0.05 vs. CS group.

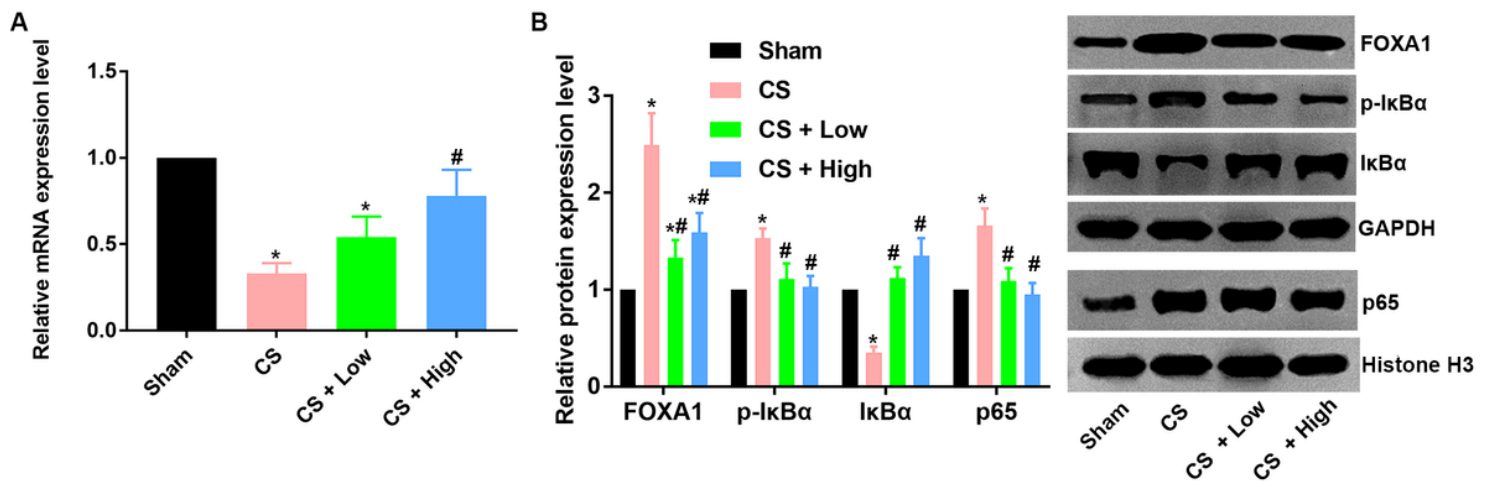


Figure 3

Effects of NS model and berberine administrations on level of miR-132-3p and activity of FOXA1 and NF- κ B pathway. The expression of miR-132-3p was detected using RT-qPCR. The expressions of FOX1, p-I κ B α , I κ B α , and p65 were detected with western blotting assays. A) Quantitative analysis results of miR-132-3p level. B) Quantitative analysis results of FOX1, p-I κ B α , I κ B α , and p65 levels. “*” represents $p < 0.05$ vs. Sham group. “#” represents $p < 0.05$ vs. CS group.

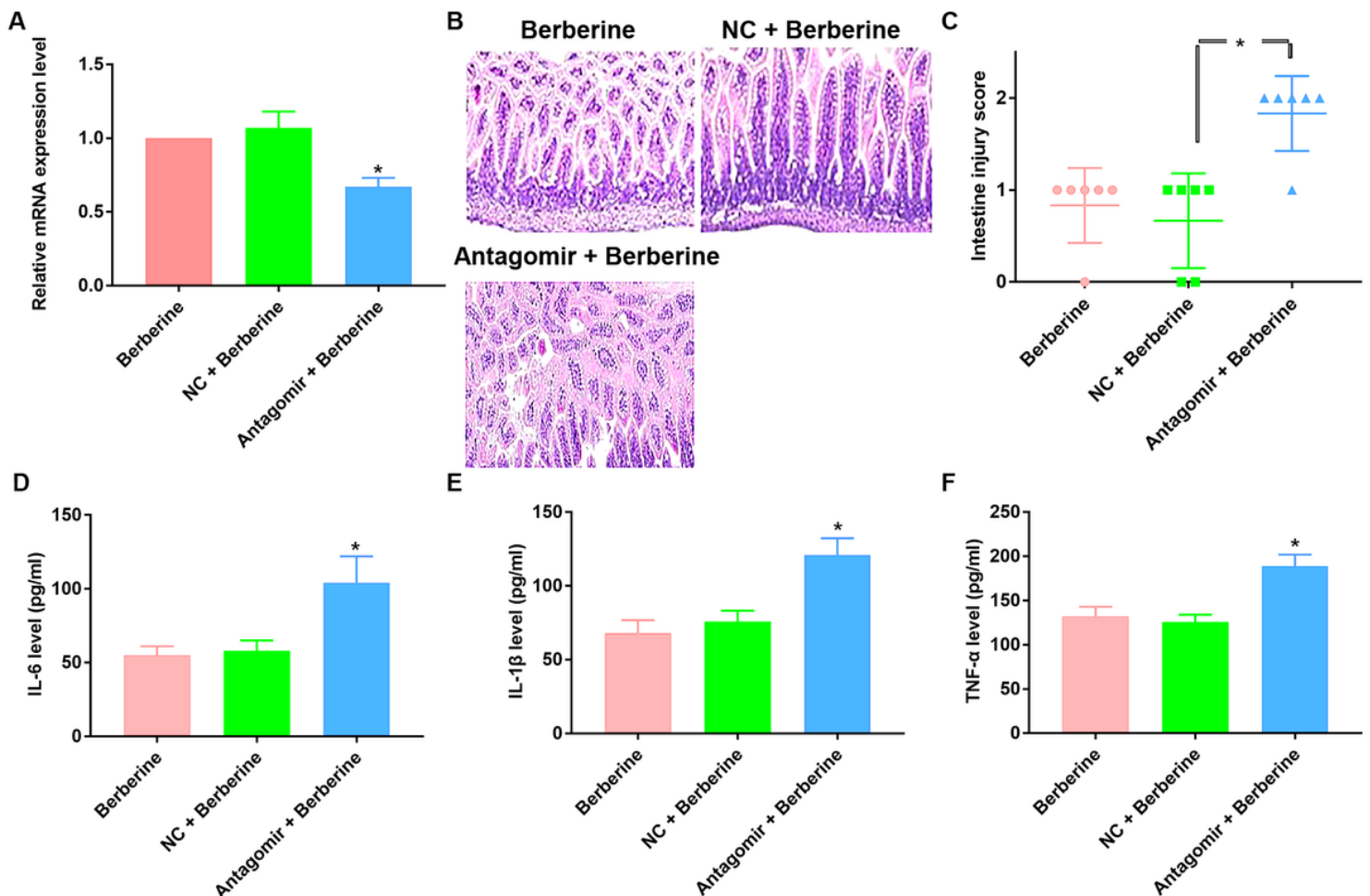


Figure 4

Effects of miR-132-3p antagomir injection on the anti-NS effects of berberine. The level of miR-132-3p was inhibited using specific antagomir 24 h prior to CS and berberine administrations. Then the changes small intestine histology and systemic inflammation were detected as described above. A) Quantitative analysis results of miR-132-3p level. B) H&E staining detection of small intestinal structure. C) Quantitative analysis results of injury score. D) Quantitative analysis results of IL-6 level. E) Quantitative analysis results of IL-1 β level. F) Quantitative analysis results of TNF- α level. “*” represents $p < 0.05$ vs. NC + berberine group.

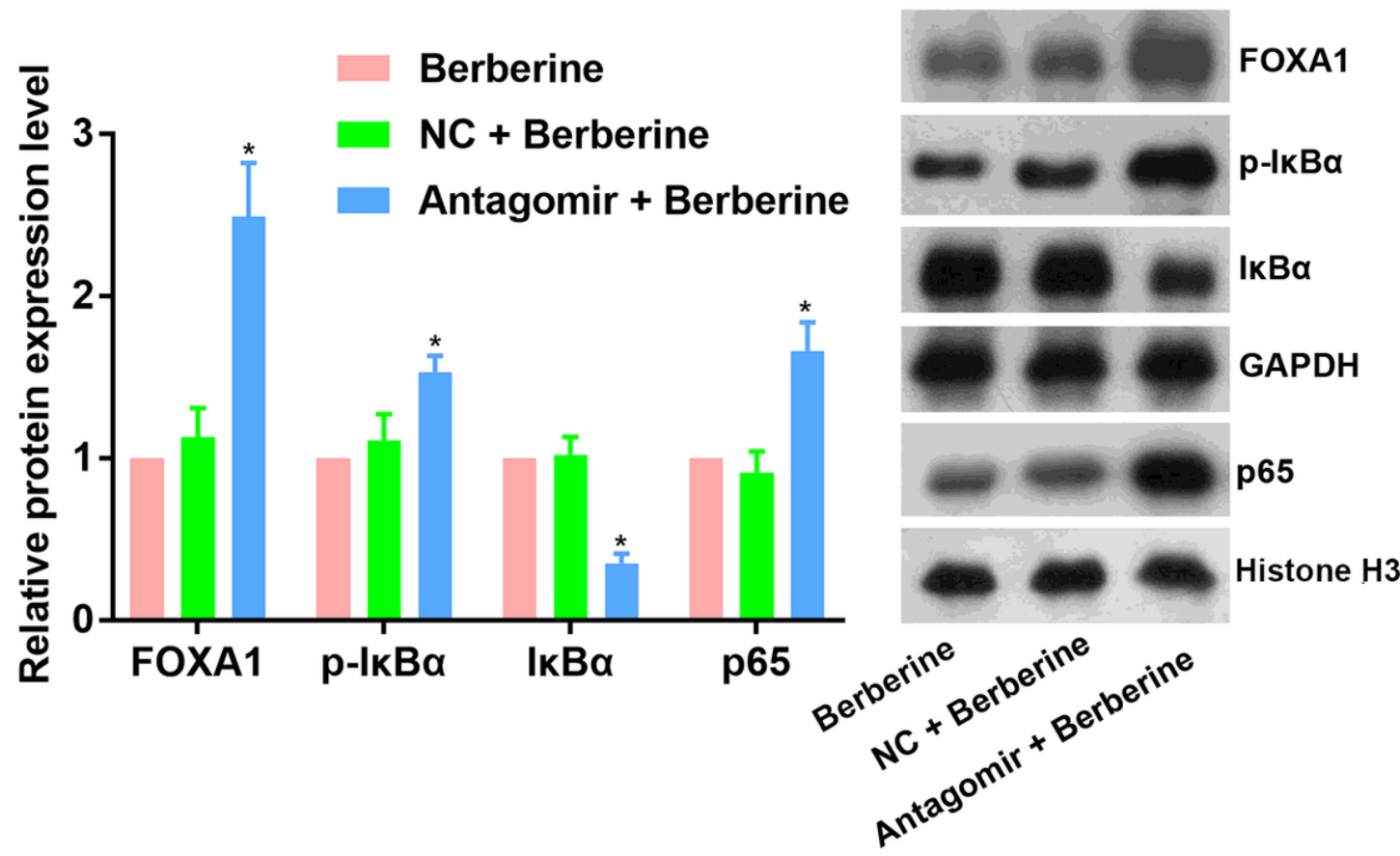


Figure 5

Effects of miR-132-3p antagomir injection on the activity of FOXA1 and NF- κ B signaling. The expressions of FOX1, p-IkB α , IkB α , and p65 were detected with western blotting assays. “*” represents $p < 0.05$ vs. NC + berberine group.

Supplementary Files

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- [FigureS1.tif](#)
- [Supplementarydata.docx](#)