RESEARCH ARTICLE

Berberine impairs coxsackievirus B3-induced myocarditis through the inhibition of virus replication and host pro-inflammatory response

Qian Dai[1] | Xiaomei He[1] | Hua Yu[1] | Ying Bai[2] | Lu Jiang[1] | Halei Sheng[1] |
Jin Peng[1] | Maolin Wang[1] | Jiang Yu[3] | Kebin Zhang[1] (ORCID)

[1]Clinical Medicine Research Center, Xinqiao Hospital, Army Medical University (Third Military Medical University), Chongqing, China

[2]Department of Endocrinology and Metabolism, Southwest Hospital, Army Medical University (Third Military Medical University), Chongqing, China

[3]Department of Outpatient, Xinqiao Hospital, Army Medical University (Third Military Medical University), Chongqing, China

Correspondence
Jiang Yu, Department of Outpatient, Xinqiao Hospital, Army Medical University (Third Military Medical University), 400037 Chongqing, China.
Email: yujiang1997@163.com

Kebin Zhang, Clinical Medicine Research Center, Xinqiao Hospital, Army Medical University (Third Military Medical University), 400037 Chongqing, China.
Email: zhangkebin12@163.com

Funding information
National Natural Science Foundation of China, Grant/Award Number: 31872634

Abstract

Berberine (BBR), an isoquinoline alkaloid isolated from *Rhizoma coptidis*, is reported to possess antiviral activity. Our previous study has shown that BBR alleviates coxsackievirus B3 (CVB3) replication in HeLa cells. However, the anti-CVB3 activity of BBR is still unclear in vivo. In this study, we explored the effect of BBR on CVB3-induced viral myocarditis in mice. These results demonstrated the beneficial effect of BBR on alleviating CVB3-induced myocarditis in vivo, which sheds new light on the utility of BBR as a therapeutic strategy against CVB3-induced viral myocarditis.

KEYWORDS
anti-CVB3 activity, berberine, coxsackievirus B3, viral myocarditis

1 | INTRODUCTION

Myocarditis, an inflammatory disease of the myocardium, is primarily caused by a viral infection.[1] Many viruses are responsible for causing viral myocarditis (VMC). Among these, coxsackievirus B3 (CVB3) is one of the most common pathogens.[2] Mechanistically, previous studies have shown that CVB3-induced myocarditis is associated with the direct damage of cardiomyocytes during viral replication.[3] In addition, accumulating evidence has demonstrated that the excessive inflammation caused by immune cells, such as macrophages, plays an important role in the progression of heart injury in CVB3-induced VMC.[4] Nevertheless, at present, the pathogenesis of CVB3-induced VMC remains incompletely understood. Even though symptomatic treatments have been widely utilized to relieve CVB3-induced myocarditis, effective strategies that can treat CVB3-induced VMC are currently unavailable.

Berberine (BBR) is an isoquinoline alkaloid that is extracted from *Rhizoma coptidis* (also named "*Huang Lian*" in Chinese).[5] It is reported that BBR has multi-pharmacological effects, such as antidiarrheal, antibacterial, anti-inflammatory, anti-fibrotic, and antioxidative effects.[6] In recent years, a growing body of studies has revealed the antiviral activity of BBR against a variety of viruses, such as chikungunya

virus (CHKV), Semliki Forest virus, Sindbis virus, enterovirus 71, herpes simplex virus (HSV), and human immunodeficiency virus (HIV).[7] In addition, our previous study has uncovered the anti-CVB3 activity of BBR in vitro.[8] But the effects of BBR on alleviating CVB3-induced myocarditis remain unknown in vivo.

To explore the antiviral effects of BBR in vivo, a mouse model with intraperitoneal infection of CVB3 was utilized. Our findings suggest that BBR increased the survival rate and heart function, whereas reducing heart injury and myocardial viral titer in CVB3-induced myocarditis mice. Meanwhile, BBR treatment inhibited macrophage infiltration and pro-inflammatory cytokines/chemokines production in CVB3-induced myocarditis in mice. Collectively, the present study evaluates the antiviral activity of BBR in vivo model of CVB3-induced myocarditis, providing a new hint of BBR as a therapeutic agent against CVB3-induced VMC.

2 | MATERIALS AND METHODS

2.1 | Virus strain, animals, and pharmacological compound

CVB3 (Nancy strain) was purchased from the Wuhan Institute of Virology, Chinese Academy of Sciences (Wuhan, China). The BBR (molecular weight 371.82, purity > 97%, Melone Pharmaceutical Co., Ltd.; Figure 1A) was dissolved in dimethyl sulfoxide (DMSO).

The 6-week-old male BALB/c mice (16–18 g) were purchased from the Animal Center of Third Military Medical University (Chongqing, China). Use and care of the animals were in accordance with protocols approved by the Animal Use and Care Committee of the Third Military Medical University.
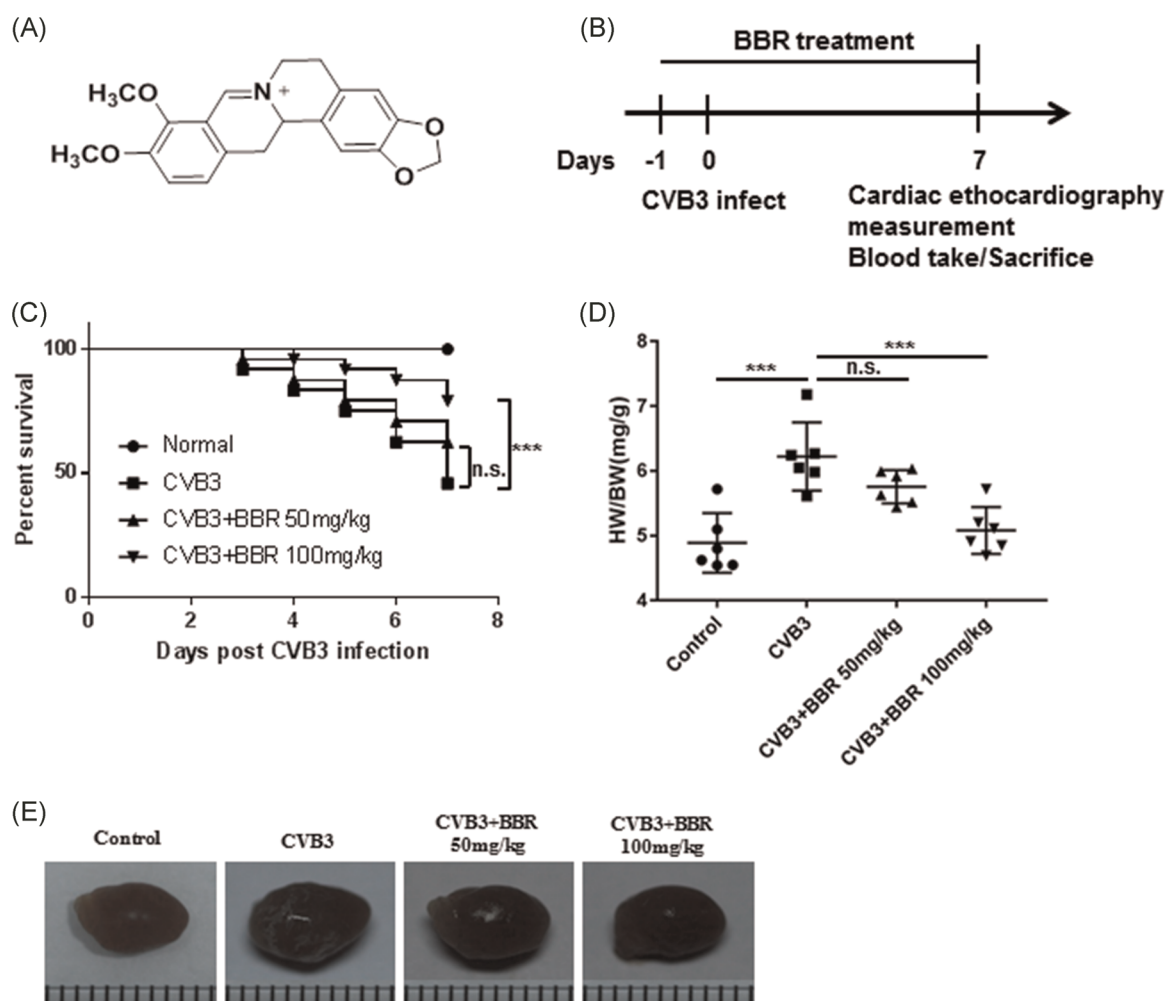

FIGURE 1 The BBR protects mice from CVB3-induced viral myocarditis. A, The chemical structure of BBR. B, Experimental protocol. One day before CVB3 infection, the mice were intragastrically administered with BBR for 7 days. On the 7th day, all experiments were measured. C, The survival rates of mouse after CVB3 infection or/and BBR treatment (n = 24). D, The measurement of mouse heart weight to body weight ratio after CVB3 infection or/and BBR treatment (n = 6). E, The representative photographs of the whole hearts. Data show mean ± SEM of mice. BBR, berberine; CVB3, coxsackievirus B3. ***$p < .001$, n.s., no significant

2.2 | Mouse models of CVB3 infection

The 6-week-old male BALB/c mice were randomly assigned to four groups containing CVB3, CVB3 + BBR (50 mg/kg), CVB3+BBR (100 mg/kg), phosphate-buffered saline (PBS), and each group consisted of 12 mice. On Day 1, the mice were intraperitoneally injected with 100 µl of 3×10^7 plaque-forming units (PFU/ml) of CVB3 diluted in PBS. Treatments were started the day before CVB3 infection. BBR (50 mg/kg/day), BBR (100 mg/kg/day) or PBS (0.5% DMSO) was treated daily by oral gavage. After 7 days, the mice were killed, following which, the hearts and spleens were harvested for subsequent analyses. For clarification, the experimental protocol is shown in Figure 1B.

2.3 | Determination of myocardial edema and lesion

To determine the presence of heart edema, whole hearts from the mice infected with CVB3 or/and treated with BBR for 7 days were removed and weighed. Mouse heart edema was evaluated by calculating the heart weight (after blood flushing) to body weight ratio (HW/BW). The heart tissues were fixed in 4% neutral buffered formalin and were then embedded in paraffin and cut into 3 µm sections. The heart tissue sections were visualized by hematoxylin and eosin (H&E) staining. The percent area of cellular infiltration and myocardial necrosis was graded in a blinded manner by 2 observers on observation of five microscopic views from each heart section, and scored as follows: 0, no lesion; 1+, lesions involving < 25%; 2+, lesions involving 25% to 50%; 3+, lesions involving 50% to 75%; 4+, lesions involving >75%. To further measure the severity of myocardial damage, the serum contents including serum lactate dehydrogenase (LDH), aspartate aminotransferase (AST), creatine kinase (CK), and creatine kinase-MB fraction (CK-MB) were determined using an automatic biochemistry analyzer Hitachi 7170S (Hitachi).

2.4 | Cardiac echocardiography analysis

The mice infected with CVB3 or underwent BBR treatment for 7 days were subjected to transthoracic echocardiographic measurement for the evaluation of mouse heart function. Cardiac echocardiography including the contents of ejection fraction (EF) and fractional shortening (FS) was performed using a Vevo 2100 high-resolution ultrasound system (Visualsonics) equipped with a 30-MHz, 100-frame-per-second micro visualization scan head.

2.5 | Immunohistochemistry and immunofluorescence staining

To direct observe virus particles in the mouse heart tissues, the heart paraffin sections were stained by immunohistochemistry (IHC) assay using a rabbit anti-CVB3 polyclonal antibody (1:100; Millipore; Cat No: 2234140) as the primary antibody. Nonimmune goat serum was used as negative control. After incubation with the indicated antibody, the tissue sections were visualized by 3,3-diaminobenzidine (Zhongshan) and observed under a light microscope (Olympus BX63).

Immunofluorescence (IF) staining was performed to identify the infiltrated macrophages in mouse myocardium by incubating the tissues with mouse anti-CD68 (1:200; Santa Cruz; Cat No: sc-52998) monoclonal antibody, followed by hybridization with Dylight 649 goat anti-mouse IgG (1:1000; Invitrogen). The nuclei of the cells were stained with 4,6-diamidino-2-phenylindole (1:1000; Beyotime). Images were acquired and analyzed using a Leica TCS SP5 laser confocal microscope.

2.6 | Flow cytometry analysis

The splenocytes from the CVB3- or/and BBR-treated mice on 7 days postinfection were isolated and suspended in PBS containing 10% fetal bovine serum (FBS). The red blood cells were lysed in red cell lysis buffer (Tiangen) for 5 min, and the remaining cells were collected and resuspended in 1% FBS/PBS at a density of 1×10^6 cells/ml. The cells were stained with cell surface marker antibodies including APC anti-mouse CD3 (Cat No: 561826), FITC anti-mouse CD4 (Cat No: 561831), and PE anti-mouse CD8 (Cat No: 561095) antibodies (BD Biosciences). After the cells had been stained at 4°C for 1 h, the samples were washed in 1% FBS/PBS and measured by flow cytometry on a FACSCalibur cell sorter (Beckman). The data were analyzed using CellQuest software.

2.7 | Measurements of cytokines and chemokines by enzyme-linked immunosorbent assay (ELISA)

The cytokines and chemokines including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, C-C motif chemokine ligand 2 (CCL2), CCL5, and C-X-C motif chemokine ligand 10 (CXCL10) in the serum samples were detected by using the corresponding ELISA kits (Westang) according to the manufacturer's instructions.

2.8 | Real-time PCR

The real-time PCR assays for analyzing the gene expression of CVB3 capsid *vp1* was applied following the manufacturer's instructions (Takara). Relative gene expression was determined by the comparative $C(t)$ ($2^{-\Delta\Delta C_t}$) method with GAPDH as the endogenous control. The primers used were as follows:

vp1 forward primer: 5′-AAACTCAGGTGCCAAGCGGT-3′
vp1 reverse primer: 5′-TTGGTGTGTTAGGATCTGTGC-3′
GAPDH forward primer: 5′-CATCAAGAAGGTGGTGAAGCAG-3′
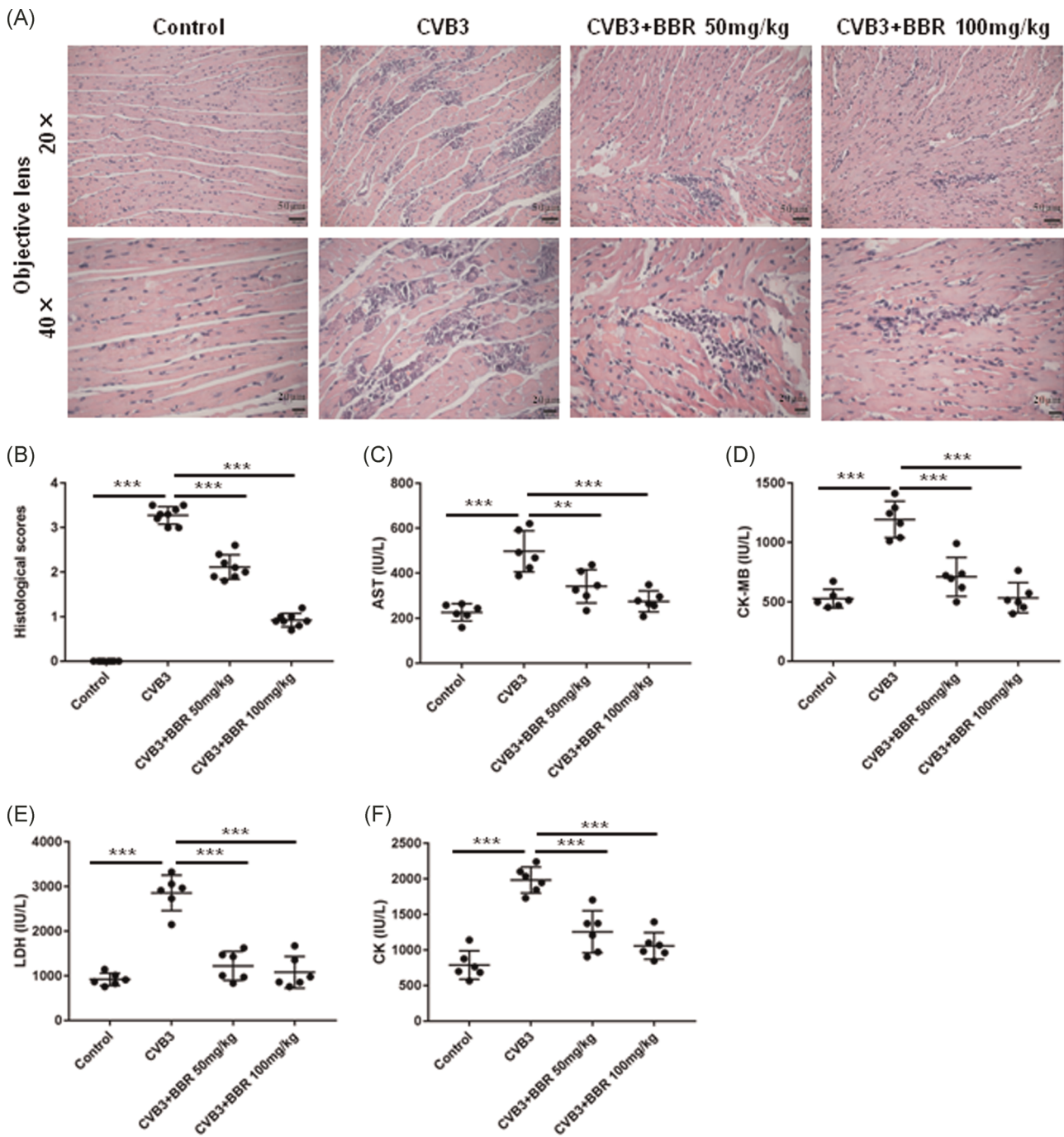GAPDH reverse primer: 5′-CGTCAAAGGTGGAGGAGTGG-3′

(A)

FIGURE 2 BBR protects mice heart lesion from CVB3-induced myocarditis mice. A, Heart histological examination by H&E staining. Upper images, scale bars = 50 μm, magnification ×200 and lower images, scale bars = 20 μm, magnification ×400. B, The heart lesion degree evaluated by blind scoring methods from H&E staining ($n = 8$). C, The myocardial zymogram containing AST, (D) CK-MB, (E) LDH, and (F) CK in mice serum were determined by an automatic biochemistry analyzer ($n = 6$). Data show mean values ± SEM of mice. AST, aspartate aminotransferase; BBR, berberine; CK, creatine kinase; CK-MB, creatine kinase-MB fraction; CVB3, coxsackievirus B3; H&E, hematoxylin and eosin; LDH, lactate dehydrogenase. **$p < .01$, ***$p < .001$

2.9 | Measurement of virus titer

Mice were killed at Day 7 postinfection. Hearts were collected under aseptic conditions and homogenized in 1 ml of PBS. Heart homogenates were subjected to three freeze-thaw cycles. The virus titers were determined by a 50% tissue culture infective dose ($TCID_{50}$) assay.[9] Briefly, HeLa cells were seeded into 96-well plates until they reached 70% confluence. A series of diluted supernatants were inoculated with HeLa cells at 37°C, 5% CO_2 for 72 h. The virus titers were calculated by the Reed-Muench method.

2.10 | Statistical analysis

Survival data were analyzed by the Kaplan-Meier method and compared with a log-rank test. The data are presented as the mean ± SEM. Statistical analysis was performed by a one-way analysis of variance followed by Tukey's post hoc test using GraphPad Prism 7. A value of p less than .05 was accepted as statistically significant.

3 | RESULTS

3.1 | BBR improves survival in CVB3-infected mice

To analyze the probable protective efficacy of BBR against CVB3 infection in mouse model, the mice that suffered from CVB3 infection were daily administrated with BBR by gavage. After BBR treatment for 7 days, the mice infected with CVB3 alone showed a 45.83% survival rate, whereas the mice treated with CVB3+BBR showed a 62.50% (BBR 50 mg/kg) and 79.17% (BBR 100 mg/kg) survival rate (Figure 1C). Even though the survival rate of CVB3+BBR (50 mg/kg)-treated mice was not statistically different from the CVB3-infected mice, the survival rate of the CVB3+BBR

(100 mg/kg)-treated mice was significantly higher than that of the CVB3-infected mice (Figure 1C), suggesting that BBR may be used as a protective reagent against CVB3 infection in vivo.

3.2 | BBR protects mice from CVB3-induced heart lesion

To further evaluate the protective effect of BBR on CVB3-infected mice, the hearts of CVB3-infected mice treated with or without BBR were harvested. The evaluation of heart edema as determined by the HW/BW ratio revealed that BBR treatment significantly reduced heart edema in CVB3-infected mice (Figure 1D,E). The analysis of heart lesion by H&E staining demonstrated that the mice infected with CVB3 suffering from BBR treatment displayed less myocardial necrosis than the CVB3-infected mice (Figures 2A,B). Given myocardial lesion might lead to increased levels of CK-MB, AST, CK, and LDH in serum, we further analyzed the contents of myocardial enzymes in the serum of the mice. Lower contents of CK-MB, AST, CK, and LDH were observed in the mice treated with CVB3+BBR than the mice that suffered from CVB3 infection (Figure 2C-F). Therefore, these results demonstrated that BBR protects mice from CVB3-induced heart damage.
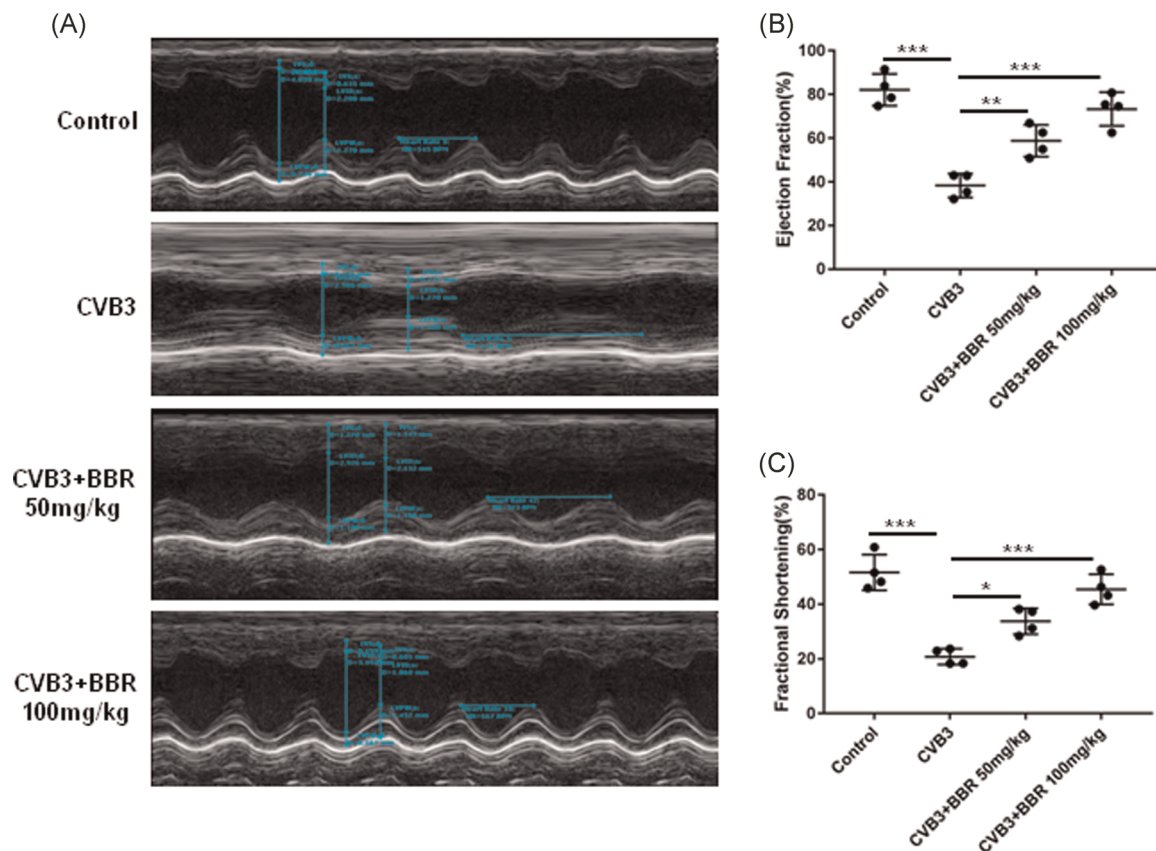

FIGURE 3 BBR protects heart function from CVB3-induced myocarditis mice. A, Echocardiogram experiments. B-C, The results of ejection fraction and fractional shortening are shown. Data show mean ± SEM of mice. BBR, berberine; CVB3, coxsackievirus B3. *p < .05, **p < .01, ***p < .001
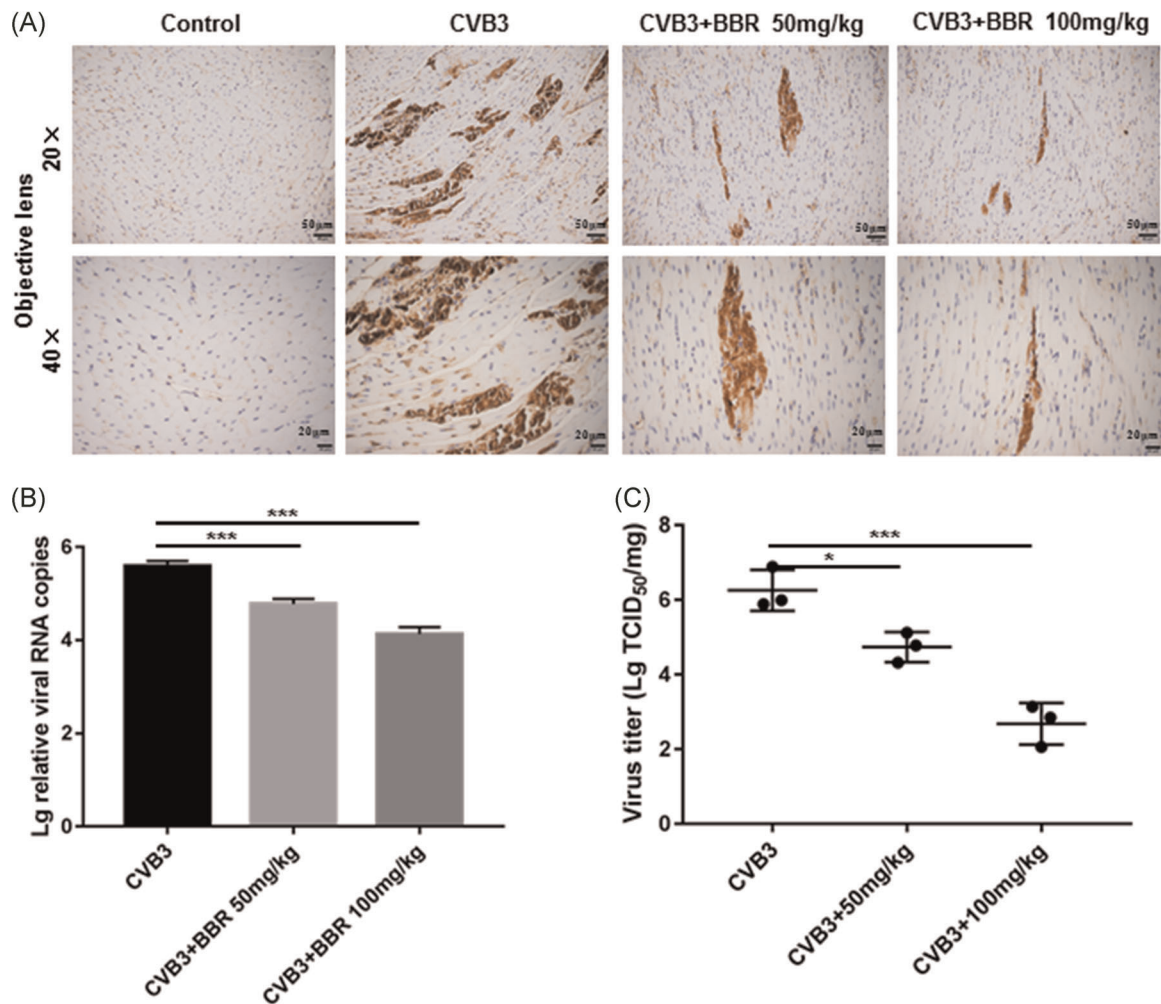
(A)

(B)

(C)

FIGURE 4 BBR inhibits titer of CVB3 in vivo. A, The IHC staining for the CVB3 particles in mouse myocardium of the indicated groups. B, The mRNA expression level of the virus *vp1* gene in mouse myocardium upon CVB3 infection or BBR treatment (*n* = 3). C, The viral titer from mouse hearts was determined by the Reed-Muench method (*n* = 3). Data show mean ± *SEM* of mice. BBR, berberine; CVB3, coxsackievirus B3; IHC, immunohistochemistry; mrna, messenger RNA. *$p < .05$, ***$p < .001$

3.3 | BBR protects heart function from CVB3-induced myocarditis in vivo

To observe the role of BBR in protecting mice from CVB3-induced heart dysfunction, the cardiac function indexes including EF and FS were evaluated by cardiac echocardiography. The CVB3-infected mice treated with BBR showed an increased EF and FS compared to CVB3-infected mice (Figure 3A-C), suggesting that BBR protects the mice from CVB3-induced heart dysfunction.

3.4 | BBR inhibits CVB3 titer in vivo

Numerous studies have suggested that CVB3 replication might directly lead to myocardium injury.[10,11] As BBR significantly impaired CVB3-induced mouse mortality and heart injury (Figures 1 and 2), we further evaluated whether the protective efficacy of BBR occurs through the direct inhibition of CVB3 replication. Results revealed

that CVB3 particles (Figure 4A) as well as *vp1* mRNA expression level (Figure 4B) in the mouse myocardium were lower in the CVB3+BBR-treated mice than that in the CVB3-infected mice. The virus titer in the heart homogenates of the CVB3+BBR-treated mice also showed a lower level than that observed in the CVB3-infected mice (Figure 4C), confirming that BBR attenuates CVB3-induced heart injury through the inhibition of virus replication.

3.5 | BBR diminishes CVB3-induced production of pro-inflammtory mediators and macrophage infiltration in vivo

Excessive inflammation plays a crucial role in the progression of virus-induced myocardial injury.[10] Therefore, in this study, we firstly investigated the levels of pro-inflammatory cytokines and chemokines in the serums of CVB3-infected mice. ELISA assay revealed that the levels of TNF-α, IL-6, IL-1β, CCL2, CCL5, and
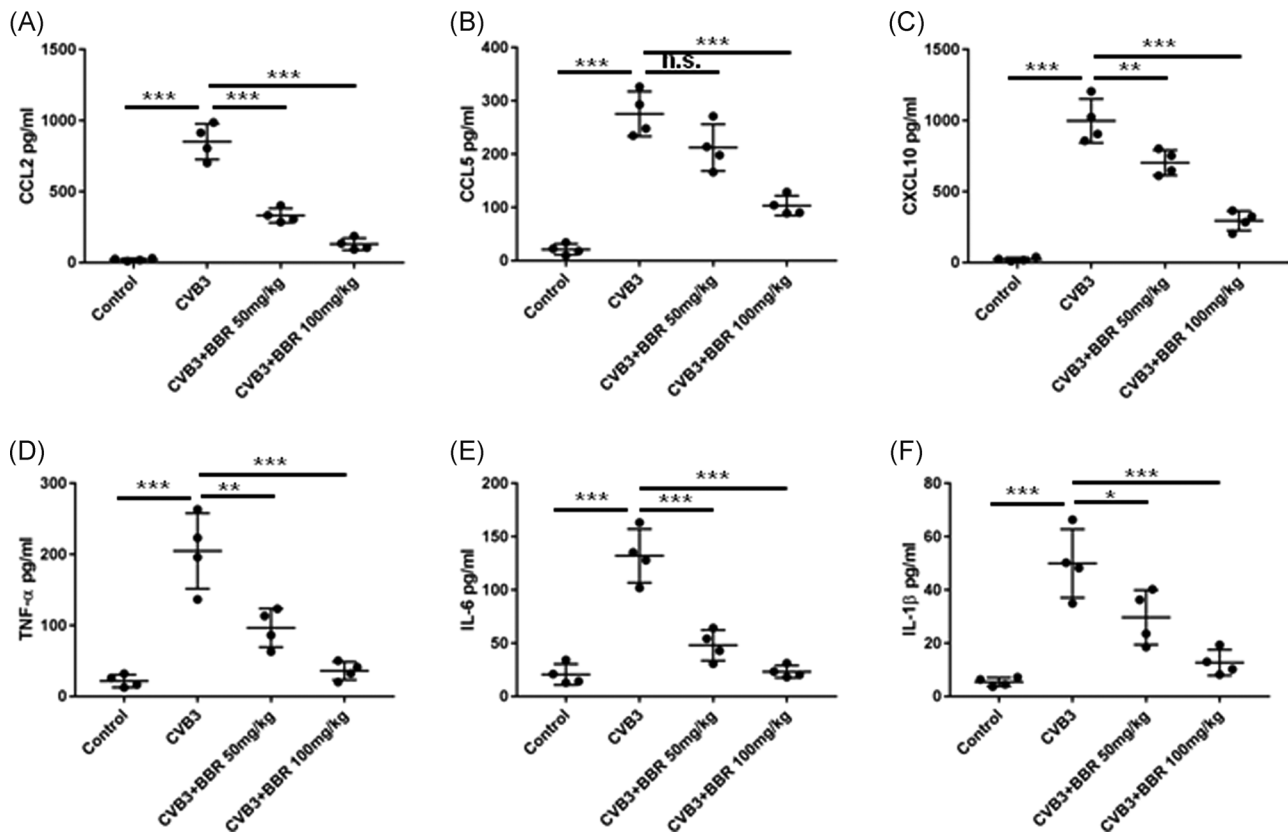
FIGURE 5 BBR diminishes CVB3-induced expression of cytokines and chemokines. The production levels of (A) CCL2, (B) CCL5, (C) CXCL10, (D) TNF-α, (E) IL-6, and (F) IL-1β in mice serum were determined by ELISA (n = 4). Data show mean ± SEM of mice. BBR, berberine; CCL2, C-C motif chemokine ligand 2; CVB3, coxsackievirus B3; CXCL10, C-X-C motif chemokine ligand 10; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α. *p < .05, **p < .01, ***p < .001

CXCL10 were significantly decreased in the serum of the CVB3+BBR-treated mice when compared to that of the CVB3-infected mice (Figure 5A-F). Given macrophage over activation is responsible for the production of the pro-inflammatory mediators as well as the promotion of inflammation-induced heart lesion upon CVB3 infection, we further analyzed the percentage of infiltrated macrophages in the heart tissues of CVB3-infected mice followed by BBR treatment. Results from IF staining showed that the percentage of infiltrated CD68+ macrophages in the myocardium of CVB3+BBR-treated mice were significantly lower than that of the CVB3-infected mice (Figure 6A), indicating that BBR decreases the infiltration of inflammation-related macrophages upon CVB3 infection.

In addition to the inhibition of inflammation response upon virus infection, activation of T cell response has also been implied to reduce CVB3-induced tissue damage. Hence, we further analyzed the percentages of CD4+ and CD8+ T cells in heart tissues upon CVB3 infection and BBR treatment. Flow cytometry analysis revealed that the proportion of CD4+/CD3+ T cells or CD8+/CD3+ T cells in the spleens of the CVB3+BBR-treated mice was comparable to that observed in the CVB3-infected mice (Figure 6B-E), indicating that BBR does not apparently alter T cell-mediated antiviral activity in CVB3-induced myocarditis.

4 | DISCUSSION

Myocarditis is often defined as an inflammatory condition of the heart. Although it can be caused by pathogens or noninfectious agents, the virus is the major cause of myocarditis.[12] A variety of viruses are associated with myocarditis in humans, including enteroviruses, adenoviruses, influenza viruses, cytomegaloviruses, parvoviruses, herpes viruses, and human immunodeficiency virus. Moreover, it has been well-known that the CVB3 is the most prevalent pathogen in causing VMC.[13,14] However, there is no specific treatment to deal with CVB3-induced myocarditis in the clinic.

It has been reported that BBR has multi-pharmacological efficacy, such as antiviral, antibacterial, and hypotensive efficacies.[15] In our previous work, we have shown that BBR exerts an antiviral effect by inhibiting the replication of CVB3 in HeLa cells.[8] Mechanistically, BBR-mediated inhibition of CVB3 has been demonstrated to be dependent on the suppression of the JNK and p38 MAPK signaling pathway. However, it remains to be further investigated whether BBR is effective in impairing CVB3-induced VMC in vivo. Therefore, we further investigated BBR's antiviral activity in vivo by using a CVB3-induced myocarditis mouse model. Here, we provided evidence showing that BBR reduces CVB3-induced mouse mortality and myocardial lesions as well as relieves heart dysfunction by lowering virus titers. Similar to
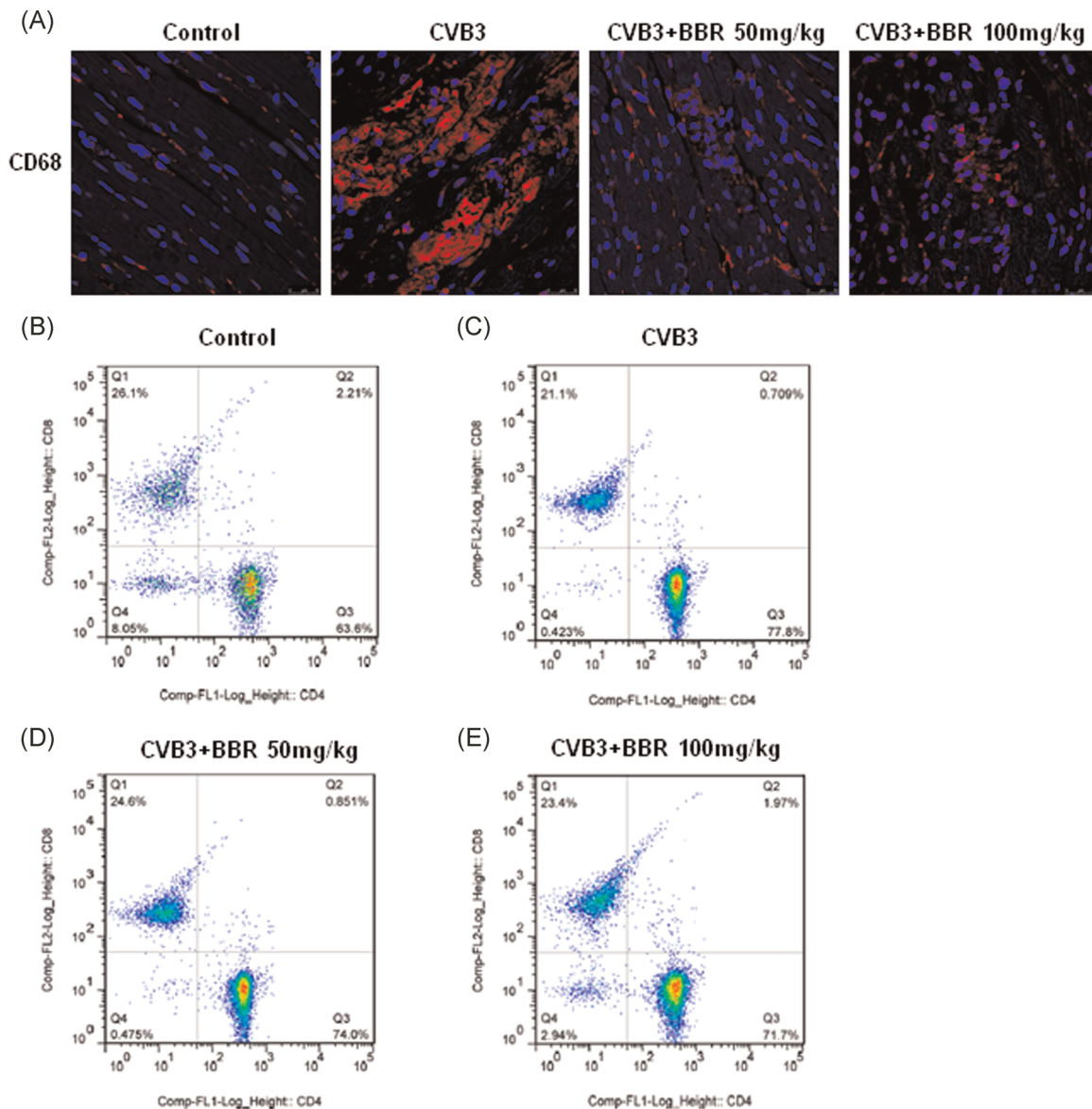
FIGURE 6 BBR attenuates the increased infiltration of immune cells in CVB3-infected mice. A, The infiltration of CD68^{+} macrophages were evaluated by immunofluorescence staining. B–E, The proportion of CD4^{+} and CD8^{+} T lymphocytes to total cells isolated from mouse spleens was determined by flow cytometry ($n = 3$). BBR, berberine; CVB3, coxsackievirus B3

our study, other researchers have discovered that 2-(3,4-dichloro-phenoxy)-5-nitrobenzonitrile reduces CVB3-induced myocarditis by decreasing CVB3 titers,[16] further proving that the reduction of virus-induced damage is vital for relieving the development of CVB3 myocarditis.

Apart from virus replication-induced tissue damage,[10,11] research have also demonstrated that the excessive inflammatory reaction-induced upon CVB3 infection can aggravate tissue injury.[17] Specifically, the sustained production of pro-inflammatory cytokines, chemokines, and recruitment of over-active immune cells contribute to the pathological changes of the CVB3-infected mouse myocardium.[18] In this study, BBR treatment has been proven to reduce the infiltration of pro-inflammatory

macrophages, and production of inflammatory cytokines and chemokines following CVB3 infection in vivo, suggesting that BBR relieves CVB3 myocarditis through the suppression of pro-inflammtory mediator generation and macrophage infiltration caused by CVB3 replication. Consistent with our findings, researchers from other groups have also demonstrated a protective efficacy of BBR against other virus-associated infection by reducing the generation of pro-inflammatory cytokines.[19,20]

In summary, our studies demonstrated that BBR exhibits protective efficacy against CVB3-induced myocarditis in mice by inhibiting CVB3 replication, production of pro-inflammatory mediators, and macrophage infiltration, indicating the therapeutic potential of BBR on attenuating CVB3 infection in vivo.

AUTHOR CONTRIBUTIONS

Qian Dai and Xiaomei He performed the experiments and drafted the manuscript; Hua Yu helped in animal experiments and data curation. Lu Jiang carried out Immunohistochemistry, immuno-fluorescence staining, and hematoxylin and eosin (H&E) staining; Halei Sheng hepled with flow cytometry analysis; Jing Peng and Maolin Wang acquired images of IF by using a confocal microscope. Kebin Zhang, Jiang Yu, and Ying Bai were involved in study design, supervision, and reviewing the manuscript. All authors approved the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Kebin Zhang http://orcid.org/0000-0003-2349-1167

REFERENCES

1. Suddaby EC. Viral myocarditis in children. *Crit Care Nurse*. 1996; 16(4):73-82.
2. Jiang D, Li M, Yu Y, Shi H, Chen R. microRNA-34a aggravates coxsackievirus B3-induced apoptosis of cardiomyocytes through the SIRT1-p53 pathway. *J Med Virol*. 2019;91(9):1643-1651.
3. Yajima T. Viral myocarditis: potential defense mechanisms within the cardiomyocyte against virus infection. *Future Microbiol*. 2011; 6(5):551-566.
4. Opitz E, Koch A, Klingel K, et al. Impairment of immunoproteasome function by β5i/LMP7 subunit deficiency results in severe enterovirus myocarditis. *PLOS Pathog*. 2011;7(9):e1002233.
5. Kong XP, Liu EYL, Chen ZC, et al. Synergistic inhibition of Acetylcholinesterase by alkaloids derived from Stephaniae Tetrandrae Radix, Coptidis Rhizoma and Phellodendri Chinensis Cortex. *Molecules*. 2019;24(24):4567.
6. Wang K, Feng X, Chai L, Cao S, Qiu F. The metabolism of berberine and its contribution to the pharmacological effects. *Drug Metab Rev*. 2017;49(2):139-157.
7. Warowicka A, Nawrot R, Goździcka-Józefiak A. Antiviral activity of berberine. *Arch Virol*. 2020;165:1-11.
8. Dai Q, Zhang D, Yu H, et al. Berberine restricts coxsackievirus B type 3 replication via inhibition of c-Jun N-terminal kinase (JNK) and p38 MAPK activation in vitro. *Med Sci Monitor*. 2017;23:1448-1455.
9. Smith PA, Rojas ME, Guajardo A, Contreras J, Morales MA, Larenas J. Experimental infection of coho salmon Oncorhynchus kisutch by exposure of skin, gills and intestine with Piscirickettsia salmonis. *Dis Aquat Organ*. 2004;61(1-2):53-7. https://doi.org/10.3354/dao061053
10. Liu H, Li M, Song Y, Xu W. TRIM21 restricts coxsackievirus B3 replication, cardiac and pancreatic injury via interacting with MAVS and positively regulating IRF3-mediated type-I interferon production. *Front Immunol*. 2018;9:2479.
11. Zheng C, Wu SM, Lian H, et al. Low-intensity pulsed ultrasound attenuates cardiac inflammation of CVB3-induced viral myocarditis via regulation of caveolin-1 and MAPK pathways. *J Cell Mol Med*. 2019;23(3):1963-1975.
12. Cooper LT Jr. Myocarditis. *N Engl J Med*. 2009;360(15):1526-1538.
13. Rivadeneyra L, Charó N, Kviatcovsky D, de la Barrera S, Gómez RM, Schattner M. Role of neutrophils in CVB3 infection and viral myocarditis. *J Mol Cell Cardiol*. 2018;125:149-161.
14. Pollack A, Kontorovich AR, Fuster V, Dec GW. Viral myocarditis—diagnosis, treatment options, and current controversies. *Nat Rev Cardiol*. 2015;12(11):670-680.
15. Liu D, Meng X, Wu D, Qiu Z, Luo H. A natural isoquinoline alkaloid with antitumor activity: studies of the biological activities of berberine. *Front Pharmacol*. 2019;10:9.
16. Padalko E, Verbeken E, De Clercq E, Neyts J. Inhibition of coxsackie B3 virus induced myocarditis in mice by 2-(3,4-dichlorophenoxy)-5-nitrobenzonitrile. *J Med Virol*. 2004;72(2):263-267.
17. Garmaroudi FS, Marchant D, Hendry R, et al. Coxsackievirus B3 replication and pathogenesis. *Future Microbiol*. 2015;10(4):629-653.
18. Han T, Zhao K, Wu C, et al. Viral kinetics are associated with changes in cytokines and chemokines in serum and target organs of SSM-CVB3-infected macaques. *Exp Mol Pathol*. 2013;94(1):174-181.
19. Wu Y, Li JQ, Kim YJ, Wu J, Wang Q, Hao Y. In vivo and in vitro antiviral effects of berberine on influenza virus. *Chin J Integr Med*. 2011;17(6):444-452.
20. Yan YQ, Fu YJ, Wu S, Qin HQ. Anti-influenza activity of berberine improves prognosis by reducing viral replication in mice. *Phytother Res*. 2018;32(12):2560-2567.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.