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Original Article

Berberine Attenuates Hepatic Sinusoidal Obstruction Syndrome by Inhibiting Endothelial-mediated Neutrophil Recruitment and Activation

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Abstract

Background and Aims: Hepatic sinusoidal obstruction syndrome (HSOS) is a life-threatening liver vascular disorder with limited treatment options. HSOS results from the activation and injury of liver sinusoidal endothelial cells (LSECs). Berberine (BBR) has been shown to protect endothelial cells in various diseases. However, whether BBR can alleviate liver injury and LSEC disruption in HSOS remains unclear. In this study, we aimed to evaluate the effect of BBR on HSOS. ***Methods:*** Two mouse models of HSOS were established using monocrotaline or oxaliplatin. Mice in the treatment groups received a low dose (100 mg/kg) or a high dose (200 mg/kg) of BBR daily. Histology, scanning electron microscopy, immunofluorescence, and flow cytometry were used to evaluate the therapeutic effects of BBR. Cell co-culture, Transwell assays, qRT-PCR, and Western blotting were performed to investigate the molecular pathways involved. ***Results:*** BBR treatment dose-dependently reduced liver injury and disruption of LSECs in murine HSOS models. Moreover, BBR significantly reduced hepatic neutrophil infiltration, thereby attenuating neutrophil-mediated injury to LSECs. Additionally, BBR inhibited the effect of injured LSECs on neutrophil activation. Mechanistically, injured LSECs were identified as one of the major sources of CXCL1 in HSOS, and BBR downregulated CXCL1 expression in injured LSECs by inhibiting MAPK signaling. ***Conclusions:*** In this study, we demonstrate that BBR ameliorates HSOS by inhibiting endothelial-mediated neutrophil recruitment and activation. BBR may be a promising therapeutic option for HSOS treatment.

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Introduction

Hepatic sinusoidal obstruction syndrome (HSOS) is a rare and life-threatening disorder affecting the hepatic sinusoids, characterized by disruption of liver sinusoidal endothelial cells (LSECs), intrahepatic congestion, portal hypertension, and liver injury.[1] HSOS is primarily caused by the use of pyrrolizidine alkaloid (PA)-containing products, oxaliplatin (OXA) chemotherapy, or hematopoietic stem cell transplantation (HSCT).[2] Current pharmacological treatments include hepatoprotective agents, antioxidants, and anticoagulants, yet therapeutic efficacy remains limited.[1,3,4] Defibrotide is the only approved drug for treating patients with HSCT-induced HSOS in the European Union and the United States. However, only approximately 50% of HSCT-HSOS patients respond to defibrotide.[5] Zhuge *et al*. reported that anticoagulation improved the prognosis of PA-HSOS patients in China, with 60% of patients responding to anticoagulation therapy.[6] The unsatisfactory pharmacological efficacy highlights the urgent need to elucidate the underlying mechanisms and develop novel treatment strategies.

Hepatic sinusoids are the primary site of injury in HSOS.[7] LSECs form the vascular wall of the hepatic sinusoids. Besides functioning as the hepatic vascular barrier, LSECs also serve as gatekeepers of liver immune homeostasis.[8] LSEC dysfunction is one of the earliest events in HSOS. Healthy LSECs help inhibit an overactive immune response; however, injured LSECs switch to a proinflammatory phenotype, exacerbating hepatic injury. Given their critical role in maintaining hepatic immune balance, preserving the healthy phenotype of LSECs has emerged as a therapeutic target for treating HSOS.

Berberine (BBR), a natural alkaloid with demonstrated hepatoprotective properties, has been shown to ameliorate drug-induced liver injury and metabolic–associated fatty liver disease (MASLD).[9,10] Additionally, BBR has been reported

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to exert protective effects on vascular endothelial cells in various diseases.[11,12] However, the effect of BBR on HSOS and LSEC protection has not been studied. In this study, we aimed to investigate the protective role of BBR in HSOS and its regulatory effect on LSEC dysfunction.

Methods

Animals and treatments

Male wild-type C57BL/6J mice aged 6–8 weeks were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. Murine HSOS models were constructed using methods described previously, and detailed procedures are provided in Supplementary File 1 (1.1). [13,14]

Cell culture and cell lines

Primary murine LSECs, Kupffer cells (KCs), hepatic stellate cells (HSCs), and hepatocytes were isolated from C57BL/6J mice using a modified method as previously described.[15] Detailed methods are provided in Supplementary File 1 (1.2).

The human liver endothelial cell line TMNK-1 was obtained from the Japanese Collection of Research Bioresources cell bank.[16] Primary human hepatic sinusoidal endothelial cells (HHSECs) were obtained from ScienCell Research Laboratories.[17] Both HHSECs and TMNK-1 cells were cultured in endothelial cell growth medium (ScienCell, 1001).

Liver histologic staining and immunofluorescence

Liver histologic staining and immunofluorescence were performed as previously described.[18] Paraffin-embedded mouse liver tissue sections were deparaffinized, hydrated, and stained. H&E staining was performed to evaluate liver tissue injury.

For immunofluorescence, paraffin-embedded mouse liver tissue sections were deparaffinized. After antigen retrieval, sections were permeabilized and blocked. Sections were then incubated with primary antibodies for 12 h, followed by incubation with secondary antibodies. Nuclei were stained with DAPI.

Total RNA extraction and real-time PCR

Real-time PCR was performed as previously described.[18] Total RNA was extracted from tissues or cells using TRIzol reagent (Invitrogen, 15596026). cDNA was synthesized, and qPCR was performed. Gene expression was calculated relative to *Gapdh*. Primer sequences are listed in Supplementary Table 1.

Flow cytometry

As described in published flow cytometry methods.[18] After mechanical dissociation of liver tissue, the pieces were filtered through a 70-μm strainer, and immune cells were isolated by 40% Percoll density gradient centrifugation. For cell surface antigen staining, single-cell suspensions were incubated with CD16/32 for 10 min at 4 °C and then stained with fluorochrome-conjugated antibodies for 30 min at 4 °C. Samples were analyzed using a BD FACSCelesta, and data were analyzed using FlowJo software (Tree Star Inc.).

Statistical analysis

All statistical analyses were performed using Prism software. Differences were analyzed using parametric (Student's t-test) or nonparametric (Mann–Whitney U or Wilcoxon) tests. One-way ANOVA and Dunnett's t-test were used for multiple comparisons. Data are expressed as mean ± standard error of the mean. A P-value < 0.05 was considered statistically significant.

More detailed information regarding the experimental procedures is included in the Supplementary File 1 (1.3–1.6). Antibodies used in immunofluorescence and flow cytometry are listed in Supplementary Table 2.

Results

BBR alleviates HSOS in mice

To investigate the protective role of BBR in HSOS, we administered monocrotaline (MCT), a PA, to establish an HSOS model in mice (Fig. 1A). In the MCT group, H&E staining showed severe endothelial cell injury, hepatic sinusoidal hemorrhage, and coagulative necrosis. Liver function parameters and proinflammatory factors were significantly elevated in the MCT group. However, in the BBR treatment group, liver necrosis, liver function parameters, and inflammation were significantly attenuated (Fig. 1B–E). We also used OXA, another common cause of HSOS,[19,20] to establish a murine HSOS model (Fig. 1F). H&E staining showed obvious endothelial cell injury and sinusoidal dilation associated with hepatocyte atrophy in the OXA group, whereas these changes were absent in the OXA+BBR group (Fig. 1G). The elevated ALT, AST, and proinflammatory factors in the OXA group were significantly reduced in the OXA+BBR group (Fig. 1H and I). Taken together, these results show that BBR significantly alleviates HSOS in two mouse models.

BBR attenuates LSEC injury in HSOS mice

Next, we explored how BBR alleviates HSOS. Given that LSEC disruption contributes to the progression of HSOS,[4] we investigated the effect of BBR on LSECs. We observed ultrastructural changes in LSECs using scanning electron microscopy. In the negative control (NC) group, fenestrae were uniformly distributed on the surface of LSECs. These fenestrae structures were disrupted in the HSOS group, characterized by a reduction in the number of normal pores and large areas of loss. However, scanning electron microscopy of liver sections from the BBR treatment group showed restoration of LSEC fenestrae and porosity (Fig. 2A and B). LYVE1 is a typical marker of healthy LSECs. HSOS mouse livers showed decreased LYVE1 expression; however, BBR treatment attenuated the loss of LYVE1 in LSECs (Fig. 2C and D). Hyaluronic acid in serum is primarily cleared by LSECs. We observed a significant increase in serum hyaluronic acid levels in the HSOS group, which was effectively counteracted by BBR treatment (Fig. 2E). These findings collectively suggest that BBR attenuates LSEC injury in HSOS mice.

BBR attenuates LSEC injury by inhibiting neutrophil infiltration in HSOS mice

We next investigated how BBR attenuates LSEC injury. Immune–endothelial cell crosstalk plays an important role in LSEC injury.[17,21,22] We therefore evaluated changes in hepatic immune cells following BBR treatment. We observed a significant increase in neutrophils and monocyte-derived macrophages (MoMCs) and a decrease in KCs in the HSOS group compared with the NC group. In the MCT+BBR group, BBR treatment significantly reduced neutrophil and MoMC infiltration, with a more pronounced reduction in neutrophils (Fig. 3A and B; Supplementary Fig. 1). However, in the OXA+BBR group, BBR treatment decreased the proportion of neutrophils but had no obvious effect on MoMCs (Fig. 3C and D). To further validate whether neutrophils can induce LSEC injury, we conducted a primary neutrophil–LSEC co-culture model *in*

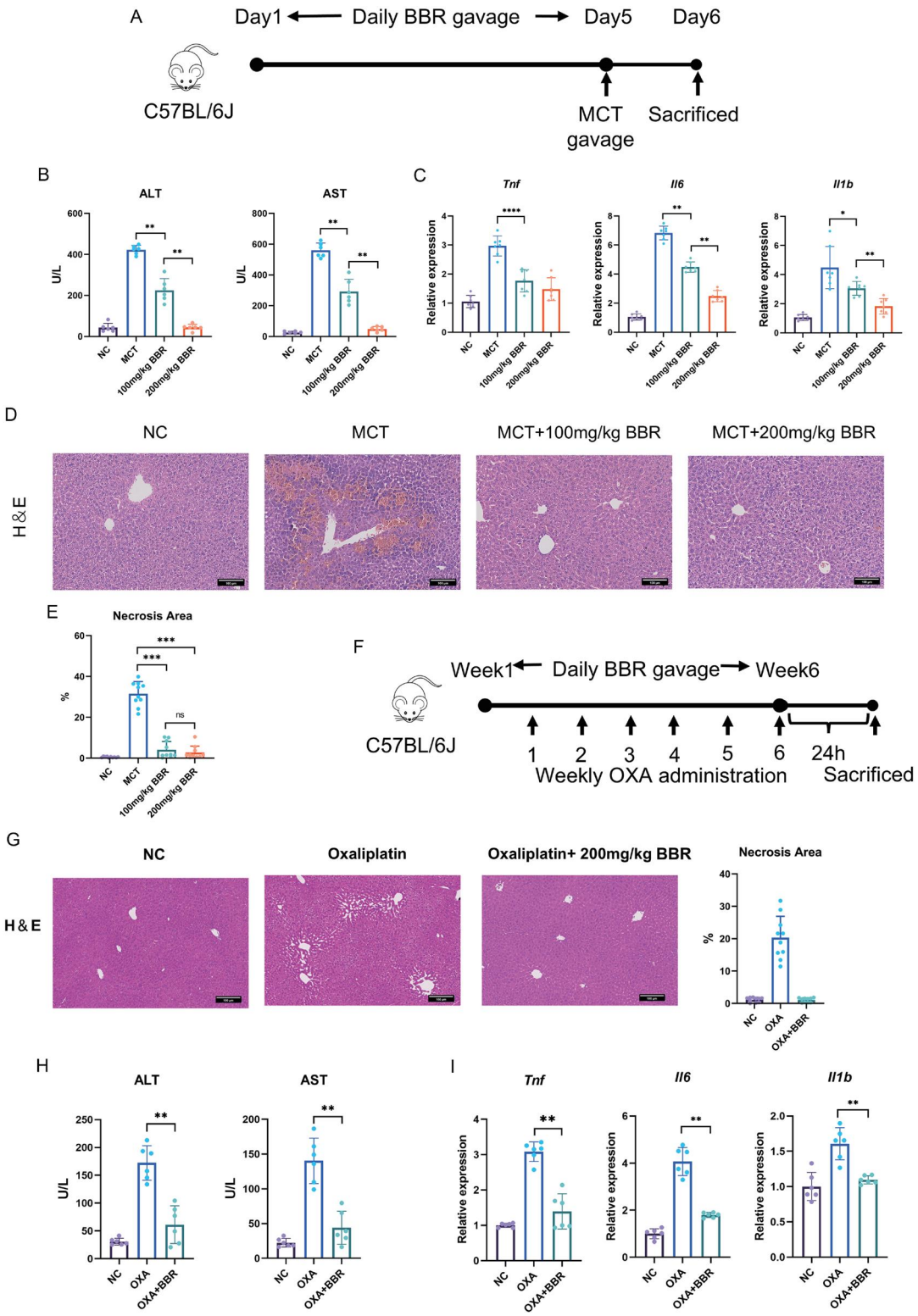

Fig. 1. The effects of BBR on two HSOS models in mice. (A) Schematic of the experimental protocol for MCT-induced HSOS. (B) Serum ALT and AST levels in the indicated groups (n = 6). (C) Relative mRNA expression of *Tnf, Il-6,* and *Il-1b* in the liver from the indicated groups (n = 6). (D, E) Representative H&E images and quantitative analysis of necrotic areas from the indicated groups. Scale bar: 100 μm. (F) Schematic of the experimental protocol for OXA-induced HSOS. (G) Representative H&E images and quantitative analysis of necrotic areas from the indicated groups. Scale bar: 100 μm. (H) Serum ALT and AST levels in the indicated groups (n = 6). (I) Relative mRNA expression of TNF-α, IL-6, and IL-1β in liver tissue from the indicated groups. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. BBR, berberine; OXA, oxaliplatin; HSOS, hepatic sinusoidal obstruction syndrome; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

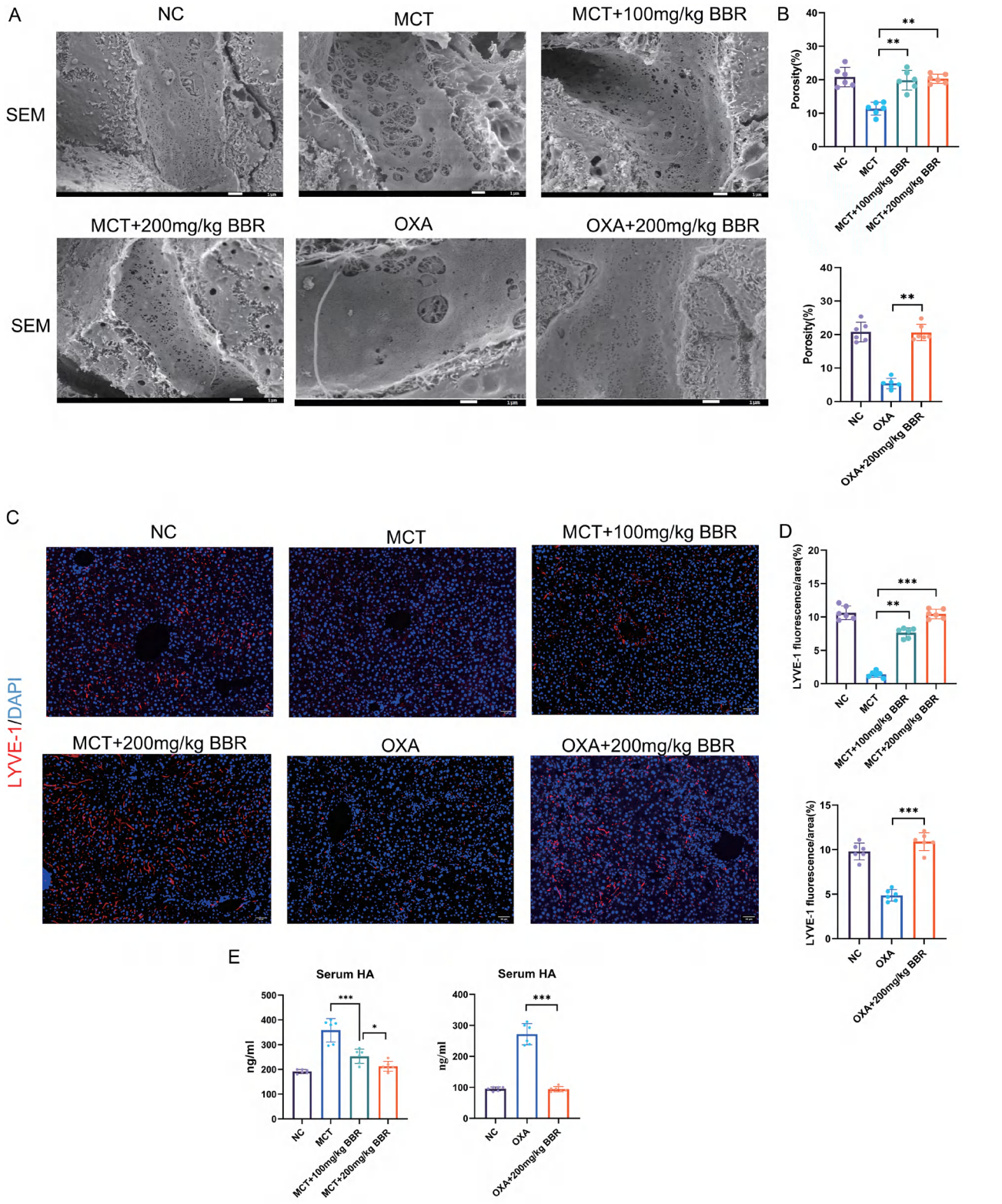

Fig. 2. The effects of BBR on LSECs in mice with HSOS. (A) Representative scanning electron microscopy (SEM) images of liver tissues from the indicated groups. (B) Quantification of LSEC porosity analyzed by SEM (n = 6). Porosity was calculated as (total fenestration area / total cell area) × 100%. (C) Representative immunofluorescence images of liver sections stained for LYVE-1 from the indicated groups. Scale bar: 50 μm. (D) Quantification of the LYVE-1-positive area (n = 10). (E) Serum hyaluronic acid (HA) levels measured by ELISA in the indicated groups (n = 6). $^{**}P < 0.01$, $^{***}P < 0.001$. BBR, berberine; OXA, oxaliplatin; LSEC, liver sinusoidal endothelial cells; HA, Serum hyaluronic acid; SEM, scanning electron microscopy.

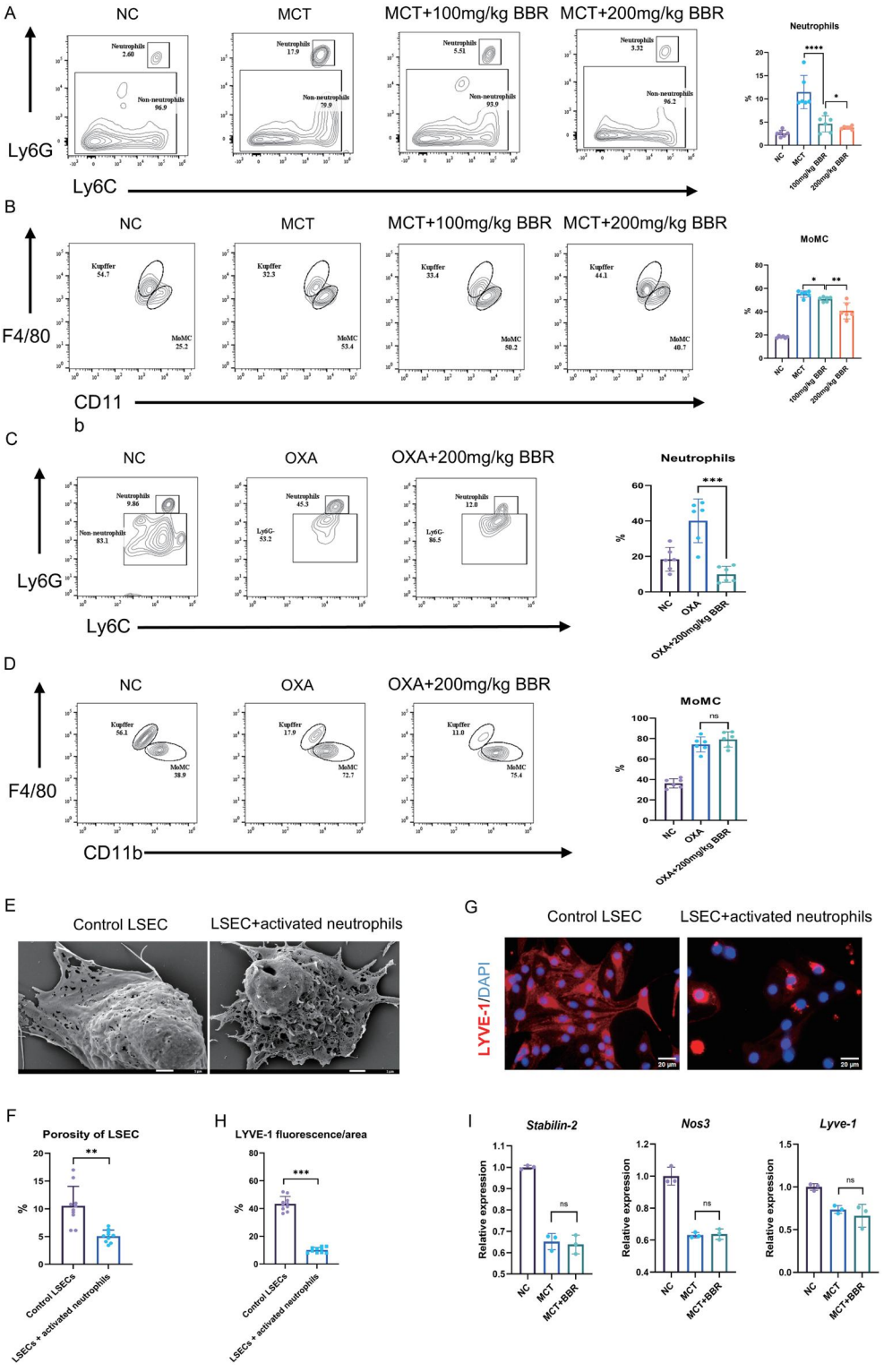

Fig. 3. The effects of BBR on hepatic neutrophil infiltration in murine HSOS models. (A) Changes in neutrophils (Ly6G^{+}, Ly6C^{+}) in MCT-induced HSOS assessed by flow cytometry. (B) Changes in Kupffer cells (F4/80^{high}, CD11b^{low}) and MoMCs (F4/80^{low}, CD11b^{high}) in MCT-induced HSOS assessed by flow cytometry. (C) Changes in neutrophils in OXA-induced HSOS. (D) Changes in Kupffer cells and MoMCs in OXA-induced HSOS. (E) Representative SEM images of primary murine LSECs cultured alone or with activated murine neutrophils. (F) Quantification of LSEC porosity analyzed by SEM (n = 10). (G) Immunofluorescence of LYVE-1 in primary murine LSECs cultured alone or with activated murine neutrophils. (H) Quantification of the LYVE-1-positive area (n = 10). (I) Relative mRNA expression in primary murine LSECs treated with MCT (2 mM) and BBR (20 μM). *P < 0.05, **P < 0.01, ***P < 0.001. ****P < 0.0001. BBR, berberine; OXA, oxaliplatin; MoMC, monocytes-derived macrophages; LSEC, liver sinusoidal endothelial cells; SEM, scanning electron microscopy.

vitro. We found obvious LSEC injury when cells were co-cultured with activated neutrophils, characterized by fenestrae disruption under scanning electron microscopy and reduced LYVE1 expression (Fig. 3E–H).

Additionally, we investigated whether BBR could directly alleviate MCT-induced LSEC injury. We found that MCT reduced the mRNA expression of Lyve1, Nos3, and Stabilin2 in LSECs *in vitro* (Fig. 3I). However, the addition of BBR did not reverse this downregulation, indicating that BBR does not directly protect LSECs from toxic injury. These findings suggest that BBR attenuates LSEC injury by inhibiting neutrophil infiltration.

BBR inhibits the expression of CXCL1 in injured LSECs

Previous studies have reported that multiple chemokines can recruit neutrophils.[23] We therefore compared the intrahepatic expression levels of these neutrophil chemoattractants following BBR treatment. In MCT-induced HSOS mice, several neutrophil chemokines were significantly upregulated, among which CXCL1 showed the highest expression level and was also the most markedly suppressed by BBR (Fig. 4A). Furthermore, immunofluorescence co-localization analysis revealed significant overlap between CXCL1 and $CD31^+$ endothelial cells (Fig. 4B). We further isolated primary hepatocytes, LSECs, macrophages (KCs), and HSCs from NC group mice, then treated them with MCT *in vitro*. Among these, macrophages and LSECs showed the highest CXCL1 expression upon MCT challenge, and BBR treatment effectively suppressed CXCL1 production in macrophages and LSECs (Fig. 4C and D).

We further validated these findings in both the human immortalized LSEC line TMNK-1 and primary HHSECs. To mimic inflammatory injury, cells were stimulated with recombinant TNF-α. TNF-α stimulation significantly increased CXCL1 expression in HHSECs, and this increase was markedly suppressed by BBR treatment (Fig. 4E). In a Transwell migration assay, primary human neutrophils were placed in the upper chamber, while TMNK-1 cells or HHSECs were seeded in the lower chamber. Results showed that neutrophils exhibited significant migration toward injured TMNK-1 cells or HHSECs. BBR treatment reduced neutrophil migration induced by injured TMNK-1 cells or injured HHSECs, but BBR in combination with anti-CXCL1 neutralizing antibody did not further decrease neutrophil migration (Fig. 4F and G). Together, these findings indicate that BBR reduces neutrophil recruitment by inhibiting CXCL1 expression in injured LSECs.

BBR inhibits neutrophil activation induced by injured LSECs

We found that myeloperoxidase (MPO) expression, a marker of neutrophil activation, was increased in MCT-HSOS mice but was significantly reduced by BBR treatment (Fig. 5A). Previous studies have shown that activated LSECs can further promote neutrophil activation.[24,25] To directly investigate this interaction, we stimulated murine primary neutrophils with lipopolysaccharide (LPS) alone or in combination with conditioned medium (CM) derived from either normal or injured LSECs (Fig. 5B). LPS stimulation alone significantly upregulated the mRNA expression of pro-inflammatory factors and increased ROS production in neutrophils. This effect was further enhanced when LPS was combined with injured LSEC-CM, whereas normal LSEC-CM did not significantly alter the LPS-induced response. Notably, BBR treatment effectively suppressed the additional elevation of both pro-inflammatory factors and ROS triggered by injured LSEC-CM (Fig. 5C and D).

To investigate the reciprocal effect, we stimulated primary LSECs with CM from activated neutrophils. Our results demonstrated that activated neutrophil-CM upregulated CXCL1 expression in LSECs. However, BBR failed to suppress this CXCL1 upregulation in LSECs induced by activated neutrophils (Fig. 5E and F). Collectively, these findings suggest that BBR mitigates neutrophil activation by suppressing the pro-activating signals from LSECs.

Inhibition of CXCL1 expression by BBR in injured LSECs involves suppression of mitogen-activated protein kinase (MAPK) activation

MAPK signaling has been reported to act as an upstream regulator of CXCL1 transcription in macrophages and hepatocytes.[26,27] Thus, we examined the effect of MAPK pathway inhibition on CXCL1 expression. In the MCT-induced HSOS model, ERK inhibitor (PD98059), JNK inhibitor (SP600125), or p38 inhibitor (SB203580) significantly reduced hepatic CXCL1 production, attenuated neutrophil infiltration, and ameliorated liver injury (Fig. 6A–D). Consistently, each MAPK inhibitor markedly suppressed CXCL1 expression in TNF-α-stimulated primary murine LSECs *in vitro* (Fig. 6E and F). Together, these results demonstrate that MAPK signaling regulates CXCL1 expression in LSECs.

We next explored whether BBR exerts its effect through modulation of MAPK activity. In MCT-induced HSOS livers, MAPK signaling was notably activated; BBR treatment significantly suppressed phosphorylation of ERK and JNK, but did not significantly affect p38 phosphorylation (Fig. 6I and J). Similarly, in TNF-α-injured primary LSECs, BBR inhibited phosphorylation of ERK and JNK without significantly altering p38 activation (Fig. 6K and L). Furthermore, when LSECs were treated with BBR in combination with either an ERK or a JNK inhibitor, BBR did not further reduce CXCL1 levels beyond inhibition by either inhibitor alone (Fig. 6G and H). These data suggest that BBR reduces CXCL1 expression in injured LSECs through inhibition of MAPK signaling.

Discussion

The current pharmacological treatments for HSOS are mainly based on anticoagulation and antithrombotic agents, in addition to supportive treatments, but their efficacy remains unsatisfactory.[1,3,5,6] Despite the pivotal role of LSEC injury in the progression of HSOS,[4,28] specific pharmacological agents aimed at protecting LSECs or interrupting their pathogenic signaling are currently lacking. Using two distinct animal models, we demonstrate that BBR attenuates murine HSOS by reducing LSEC-mediated neutrophil recruitment and activation, thereby blocking the vicious cycle between injured LSECs and neutrophils. As a widely used clinical drug with a favorable safety profile, BBR may be a promising candidate for HSOS treatment.

Although BBR has shown protection in MASLD and fibrosis through targeting hepatocytes and HSCs,[29,30] our work reveals LSECs as a new cellular target, broadening the mechanistic understanding of BBR. LSECs implicated in HSOS also play a role in other liver disorders such as MASLD and fibrosis.[31,32] Our findings also provide a novel mechanistic perspective on BBR's potential application in other liver diseases involving LSEC dysfunction.

In this study, we observed that BBR selectively suppressed ERK and JNK activation, but not p38, in injured LSECs. However, pharmacological inhibition of p38 also reduced hepatic CXCL1 levels and neutrophil infiltration *in vivo*. This apparent discrepancy may stem from BBR's selective engagement of upstream kinases specific to the ERK and JNK pathways, or from the possibility that the effects of p38 inhibition *in vivo* are indirect. Given that inhibition of each individual MAPK

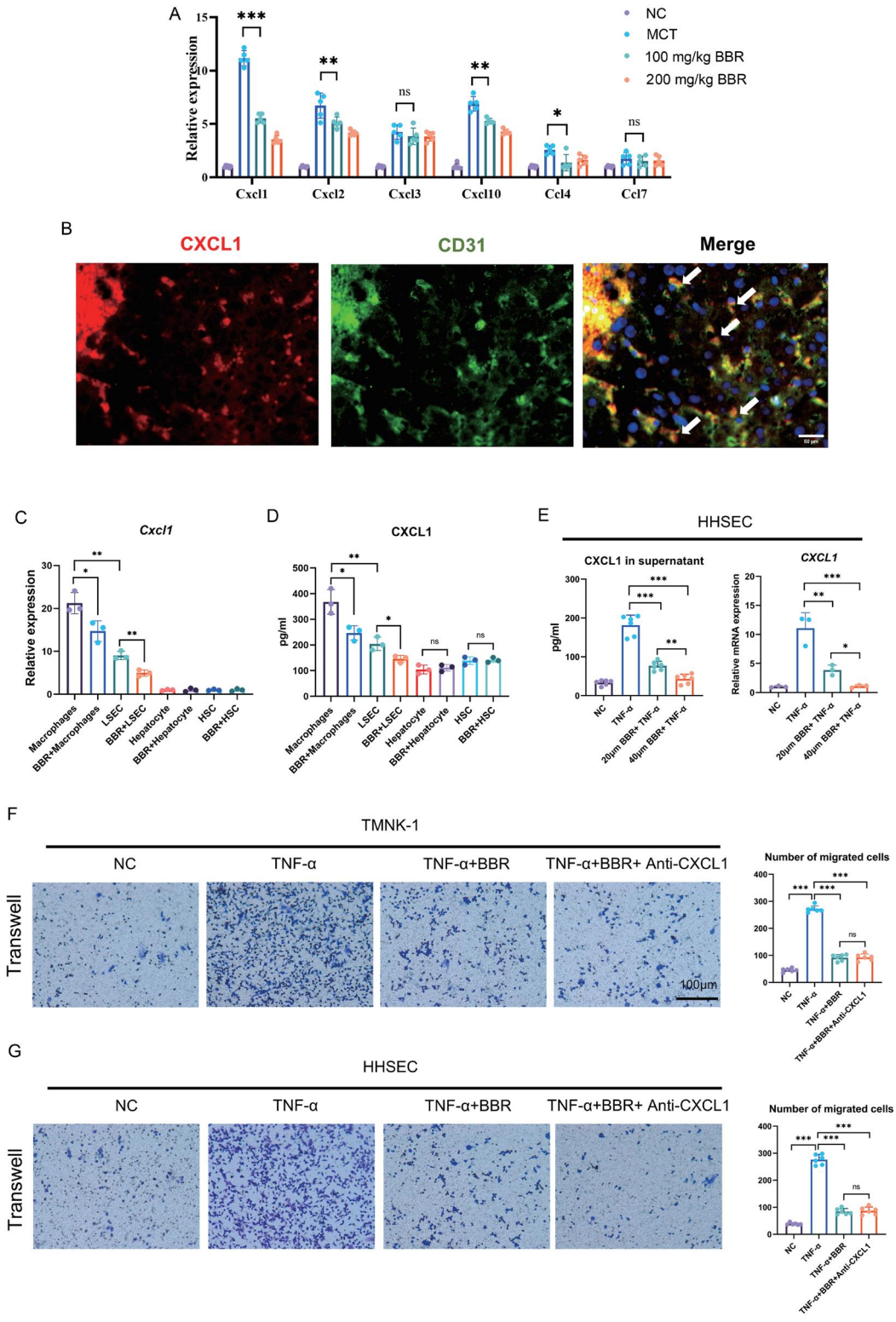

Fig. 4. The effects of BBR on CXCL1 secretion in injured LSECs. (A) Relative mRNA expression of several neutrophil chemokines in MCT-induced HSOS mice (n = 6). (B) Representative immunofluorescence images of CXCL1 (red) and CD31 (green) co-staining in MCT-HSOS liver sections. Scale bar = 50 μm. (C, D) CXCL1 mRNA levels and CXCL1 protein levels in culture supernatants in the indicated primary liver cell types following treatment with MCT or MCT + BBR. (E) CXCL1 mRNA levels and CXCL1 protein levels in culture supernatants in HHSECs treated with TNF-α or TNF-α + BBR. (F–G) Representative Transwell images of primary human neutrophil migration toward TMNK-1 cells or HHSECs treated with TNF-α, TNF-α + BBR, or TNF-α + BBR + anti-CXCL1 (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001. BBR, berberine; MCT, monocrotaline; HHSECs, human hepatic sinusoidal endothelial cells.

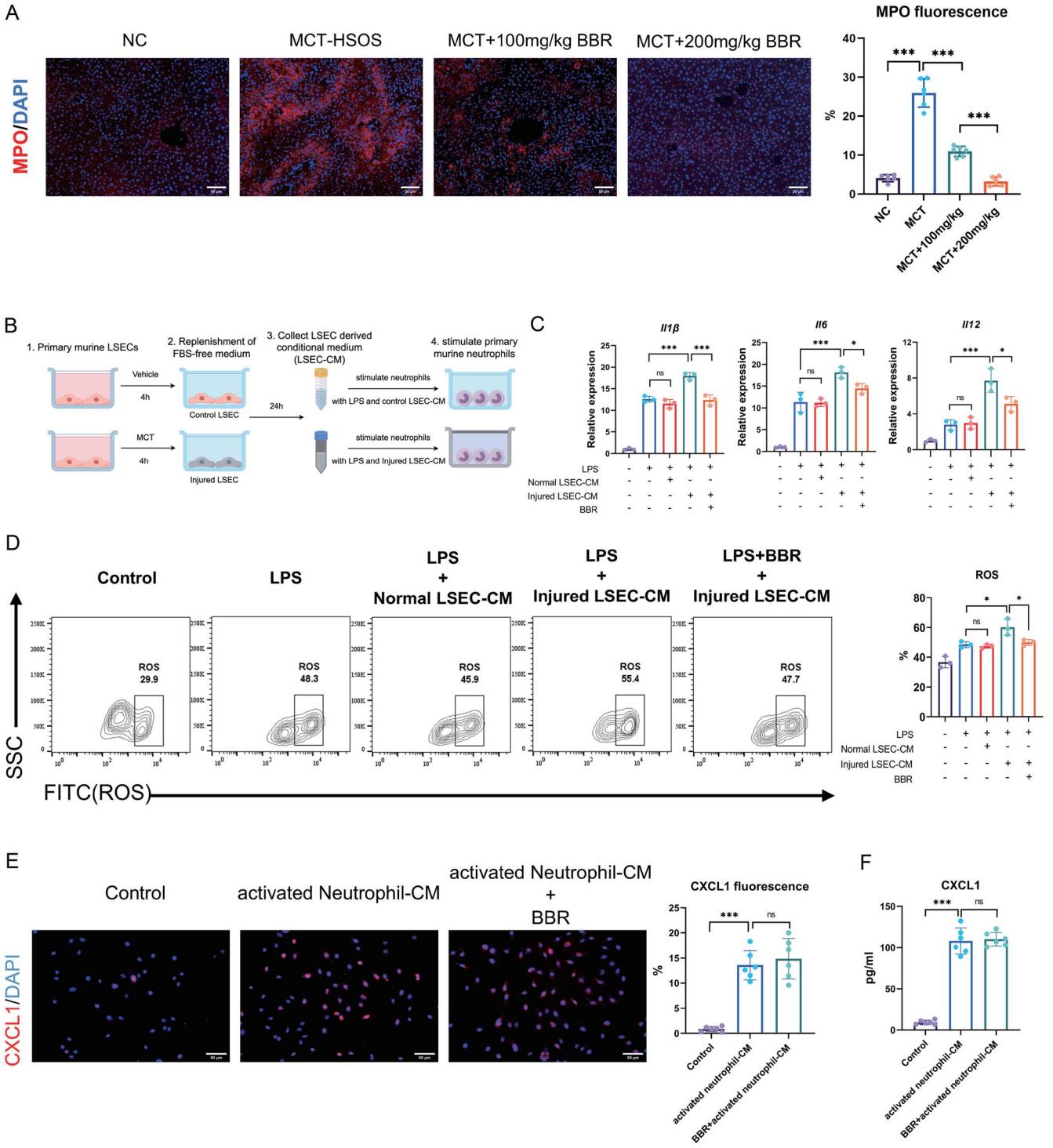

Fig. 5. The effect of BBR on neutrophil activation induced by injured LSECs. (A) Representative immunofluorescence staining of MPO in liver tissues (left) and quantification of MPO fluorescence intensity (right) across the indicated groups (n = 6). Scale bar: 50 μm. (B) Schematic diagram illustrating the experimental procedure for stimulating primary murine neutrophils with LPS in combination with conditioned medium derived from either normal or injured LSECs. (C) Relative mRNA expression of IL-1β, IL-6, and IL-12 in primary murine neutrophils treated with LPS alone, LPS combined with normal or injured LSEC-CM, or LPS combined with injured LSEC-CM plus BBR (20 μM) (n = 3). (D) Levels of ROS measured by flow cytometry in primary murine neutrophils treated with LPS alone, LPS combined with normal or injured LSEC-CM, or LPS combined with injured LSEC-CM plus BBR (20 μM) (n = 3). (E) Immunofluorescence detection and quantification of CXCL1 in LSECs stimulated with activated neutrophil-CM in the absence or presence of BBR (20 μM) (n = 6). (F) ELISA results of CXCL1 in primary murine LSECs stimulated with activated neutrophil-CM in the absence or presence of BBR (20 μM) (n = 6). **P* < 0.05, ****P* < 0.001. +, addition of the reagent; −, no addition of the reagent; BBR, berberine; MCT, monocrotaline; MPO, myeloperoxidase; LSEC-CM, liver sinusoidal endothelial cell-derived conditional medium; Neutrophil-CM, neutrophils-derived conditional medium; ROS, reactive oxygen species.

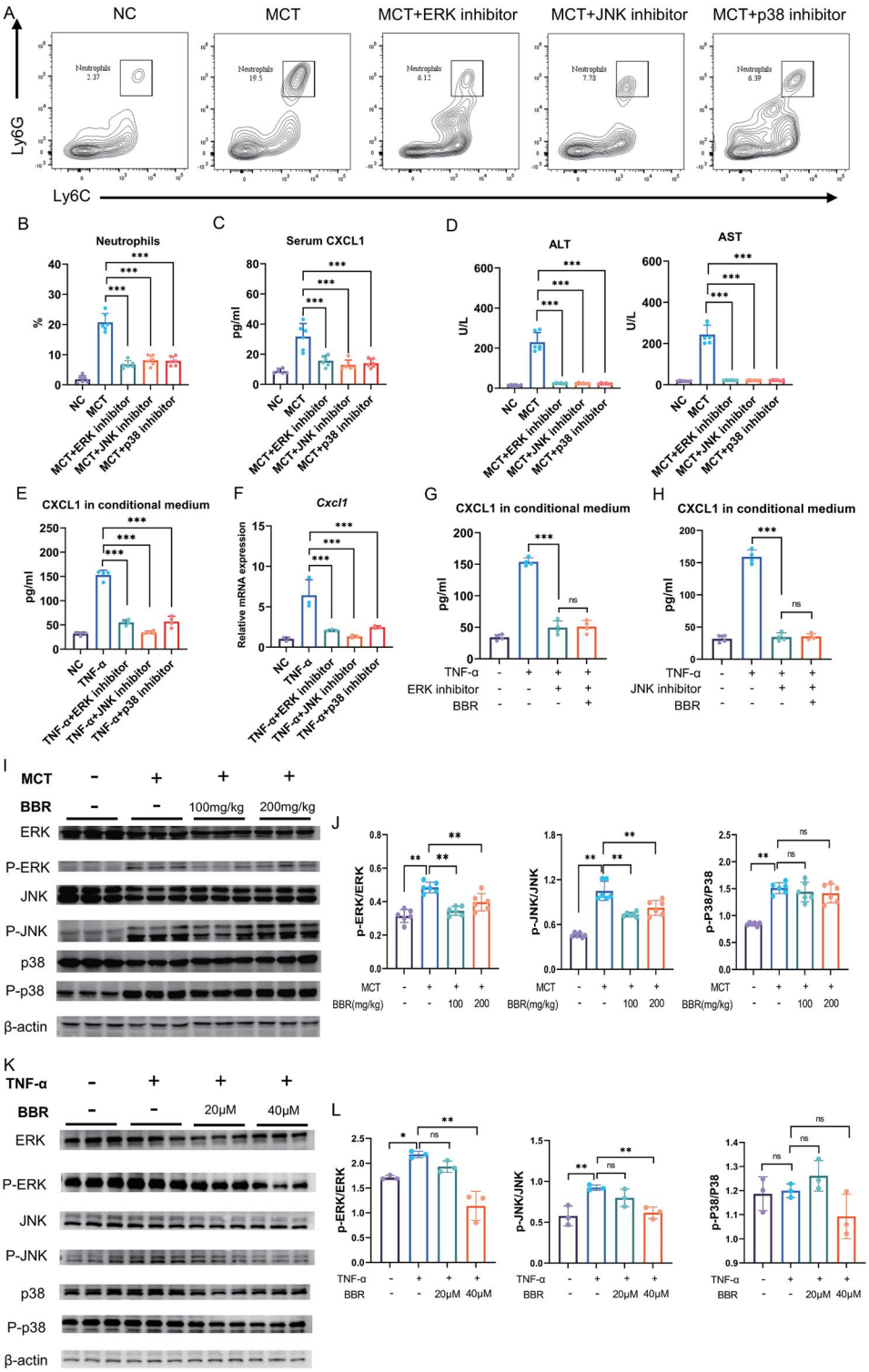

Fig. 6. The effects of BBR on MAPK signaling. (A–B) Representative flow cytometry images (A) and quantitative analysis (B) of hepatic neutrophil proportion in mice from the indicated groups (n = 6). (C–D) Quantification of serum CXCL1, ALT, and AST levels in mice from the indicated groups. (E–F) CXCL1 levels in conditioned medium and CXCL1 mRNA expression in primary murine LSECs treated with TNF-α or TNF-α combined with each MAPK inhibitor. (G) CXCL1 levels in conditioned medium of primary murine LSECs treated with NC, TNF-α, TNF-α + ERK inhibitor, or TNF-α + ERK inhibitor + BBR. (H) CXCL1 levels in conditioned medium of primary murine LSECs treated with NC, TNF-α, TNF-α + JNK inhibitor, or TNF-α + JNK inhibitor + BBR. (I, J) Representative Western blot images and quantitative analysis of MAPK pathway-related proteins in liver tissues from the indicated groups (n = 6). (K, L) Representative Western blot images and quantitative analysis of MAPK pathway-related proteins in primary murine LSECs from the indicated groups (n = 3). Data are presented as mean ± standard error of the mean. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. +, addition of the reagent; −, no addition of the reagent; BBR, berberine; MCT, monocrotaline; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

branch significantly reduced CXCL1 expression and neutrophil recruitment, the concurrent suppression of both the ERK and JNK pathways by BBR is therefore sufficient to produce these effects. This pathway selectivity of BBR is consistent with previous reports that BBR can directly inhibit MEK1/2 and MKK4/7 (upstream of ERK and JNK, respectively) but not MKK3/6 (upstream of p38), and shows a higher affinity for the JNK upstream kinase EIF2AK2 than for the p38 regulator VPS4B.[33–36] Collectively, these previous findings suggest that BBR targets multiple nodes upstream of ERK and JNK while sparing key activators of the p38 cascade, providing a plausible explanation for the selective MAPK inhibition observed. Furthermore, while pharmacological inhibition of ERK, JNK, and p38 in LSECs directly establishes that MAPK signaling regulates CXCL1 expression and that BBR selectively suppresses this pathway, BBR's multi-target nature means additional pathways may also contribute to its suppression of CXCL1.[29]

As a preclinical study, our work inevitably lacks direct clinical evidence. To bridge this gap and strengthen translational plausibility, we validated that BBR inhibits CXCL1 expression and thereby reduces neutrophil migration in both primary and immortalized human LSECs. These results suggest that the protective mechanism is conserved in human cells, providing a stronger rationale for future clinical investigation. Ultimately, prospective clinical trials will be essential to evaluate the efficacy and safety of BBR in HSOS patients.

In summary, our study elucidates a pathogenic "LSEC–neutrophil loop" in HSOS and identifies the widely used drug BBR as a mechanism-based candidate that disrupts this loop by inhibiting the MAPK–CXCL1 axis in LSECs. This study provides preclinical evidence for the future investigation of BBR in human HSOS.

Conclusions

BBR could alleviate HSOS via targeting the LSEC–neutrophil axis. BBR inhibits the recruitment and activation of neutrophils triggered by injured LSECs through inhibition of MAPK signaling.

Supporting information

Supplementary material for this article is available at 10.14218/JCTH.2026.00024.

Acknowledgments

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Conflict of interest

The authors have no conflicts of interest related to this publication.

Author contributions

Conceived and designed the study (YuL, HH), performed the experiments, analyzed the data, wrote the paper (YiL, WT), and assisted in improving the manuscript (WD, NC). All authors have approved the final version and publication of the manuscript.

Ethical statement

Animal experiments were approved by the Animal Ethics Committee of Peking University People's Hospital (Ethical approval code: 2020PHE074) and conducted in accordance with institutional and national guidelines for the care and use of laboratory animals. All animals received human care. The studies involving human subjects were performed in accordance with the Declaration of Helsinki (as revised in 2024), and were approved by the Ethics Committee of Peking University People's Hospital (Ethical approval code: 2024PHB115-001). Written informed consent was waived due to use of anonymized human-derived samples. Commercially obtained human cell lines/primary cells were used in accordance with the suppliers' instructions.

Data sharing statement

The datasets used in the study are available from the corresponding author upon reasonable request.

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