Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Berberine inhibits low shear stress-induced vascular endothelial inflammation via decreasing phosphorylation of Akt and IRF3

Yifei Lv
Nanjing Medical University
Hongfeng Yang
the Affiliated People's Hospital of Jiangsu University
Peng Ye
Nanjing Medical University
Zhiyuan Qian
Nanjing Medical University
Dongchen Wang
Nanjing Medical University
Chaohua Kong
Nanjing Medical University
Yue Gu
Nanjing Medical University
Wenying Zhou
Nanjing Medical University
Shaoliang Chen
Nanjing Medical University
Linlin Zhu
lvyifeijob@sina.com

Nanjing Medical University

Research Article

Keywords: Low shear stress, berberine, Akt, IRF3, endothelial inflammation

Posted Date: April 19th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1560144/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Tissue and Cell on December 1st, 2022. See the published version at https://doi.org/10.1016/j.tice.2022.101946.

Abstract

Background

Low shear stress (LSS) is closely related to vascular endothelial inflammation and the development of atherosclerosis. Berberine (BBR), a natural compound isolated from Coptis chinensis, has been reported to exert anti-inflammatory and anti-atherosclerotic effects. However, the role of berberine in low-shear stress-induced endothelial inflammation remains unclear.

Methods

The role of berberine in low shear stress-induced vascular endothelial inflammation was investigated in human umbilical vein endothelial cells (HUVECs) using a plate flow chamber in vitro and in mice with an established LSS model by partial ligation of the carotid artery in vivo.

Results

Firstly, in vitro experiments demonstrated that BBR significantly decreased the expression of vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) and the phosphorylation of Akt in HUVECs induced by low shear stress.Meanwhile,BBR significantly inhibited low shear stress induced IRF3 phosphorylation and nuclear translocation. Notably, Akt overexpression markedly reversed the inhibitory effects of BBR on LSS-induced IRF3 activation and ICAM-1 expression. Moreover, in vivo experiments showed that BBR markedly decreased intimal ICAM-1 and IRF3 in the LSS areas of partially ligated carotid arteries in mice; however, EC-specific Akt overexpression mediated by adeno-associated virus abolished the anti-inflammatory effect of BBR.

Conclusion

Taken together, our findings suggest that BBR treatment attenuates LSS-induced vascular endothelial inflammation by decreasing activation of the Akt/IRF3 signalling pathway.

1. introduction

Vascular endothelial inflammation induced by a low shear force is an initial and critical step in the progression of atherosclerosis and plays a role in promoting the development of atherosclerosis[1]. Atherosclerotic lesions tend to form near branch points, the outer wall of bifurcations, and the inner wall of curvatures, where disturbed flow occurs, with notably low shear stress (LSS)[2–4]. Shear stress is tangential stress caused by the friction between blood flow and vascular endothelial cells, which is closely related to blood flow velocity, properties, and lumen morphology[5]. Unidirectional laminar flow

(15-70dyne/cm^2) is proved to be anti-inflammatory and anti-atherosclerotic, whereas LSS(< 10dyne/cm^2) promotes atherosclerosis by upregulating various endothelial inflammatory genes, such as vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), and E-selectin [6, 7]. Nevertheless, effective methods for inhibiting LSS-induced inflammation are still lacking.

Akt kinase plays important roles in cell growth, migration, proliferation and differentiation[8]. Existing literature demonstrates that laminar shear stress induces PI3K-mediated Akt activation, which can reduce endothelial cell apoptosis, increase eNOS activity, and hyaluronic acid synthesis, exerting an endothelial protective role[9, 10]. However, the mechanisms and roles of Akt activation in low-shear stress reactions are quite different. Our previous study revealed that the activation of Akt is positively regulated by IKKε under LSS, but not by mTORC2 and PI3K.Notably, LSS induces IKKε-mediated Akt activation, leading to vascular endothelial inflammation by promoting IRF3 phosphorylation and nuclear translocation[11]. Although the effect of Akt/IRF3 in vascular endothelial inflammation induced by LSS has been clarified, effective interventions for this pathological mechanism are still unknown and require further investigation.

Berberine (BBR), a member of the protoberberine alkaloid group, is well known for its anti-inflammatory and antimicrobial effects on the digestive system. Recent studies have found that berberine improves endothelial function by decreasing inflammatory factors and cytokines, increasing eNOS/NO expression, and reducing oxidative stress, implying its anti-atherosclerotic role[12].Furthermore, berberine has been shown to protects against diabetes-related retinal endothelial dysfunction by inhibiting Akt activation[13].Our previous study showed that BBR attenuates LSS-induced hyaluronic acid degradation by increasing AMPKα phosphorylation [14]. However, the effects of BBR on Akt/IRF3 activation and endothelial inflammation induced by low shear stress remain unclear. In the present study, we demonstrated that BBR decreased vascular endothelial inflammation induced by low shear stress by suppressing the Akt-mediated phosphorylation of IRF3.

2. materials And Methods

2.1. Drug treatment

Berberine chloride(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium) was purchased from Sigma Chemical Co. (St. Louis, MO,USA) and dissolved in dimethyl sulfoxide (DMSO) (MP Biomedicals, LLC, France) prior to use. The cells were pretreated with or without berberine (0.01mM) for 24 h and then subjected to low-shear stress stimulation for a certain time and protein or RNA extraction.

2.2. Cell culture and treatment

HUVECs were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The cells were incubated in DMEM (Gibco) containing 10% fetal bovine serum (Gibco), 100U/mL penicillin and 100μg/mL streptomycin at 37°C and humidified with 5% CO_2. When the cells grew to 80–90% fusion, they were pretreated with or without berberine for 24 h and then subjected to low shear stress stimulation.

Radioimmunoprecipitation assay (RIPA) buffer was used to lyse the cells with protease and phosphatase inhibitors.

2.3. Animals and ethics statements

All animal experiments were performed under specific pathogen-free barrier conditions, in accordance with the National Institutes of Health Guide. All study protocols were approved by the Ethics of Animal Experiments of Nanjing Medical University. All experimental procedures were performed in accordance with the guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). All 8-week-old male C57BL/6 mice were purchased from the Animal Center of Nanjing Medical University.

The mice were randomly divided into four groups, with six mice per group. 8-week-old C57BL/6 mice were injected with AAV9-TIE-control vectors or AAV9-TIE-Akt1 vectors at a volume of 100 μL from the tail veins. The pAAV9-TIE-Akt1 vector with a genome titer of 2×10^{12} viral genomes per milliliter was generated by inserting Akt1 into the pAAV9-TIE-MCS-EGFP plasmid. A partial ligation model of the left common carotid artery with a diameter of 150μm was performed at 1 mm from the bifurcation of the carotid artery 4 weeks after virus injection. Subsequently, the mice were gavaged daily with berberine dissolved in normal saline (100 mg/kg) or the same volume of normal saline for eight weeks. The LSS fragment near the ligation site was implanted into the OCT solution for fluorescence experiments. The internal curvature of the aortic arch was separated and fixed with 4% paraformaldehyde at the time of euthanization. OCT-embedded tissue sections were prepared and transferred to slides for immunofluorescence staining.

2.4. Flow apparatus

A parallel plate flow chamber system(Naturethink, Shanghai, China) controlled by a directional controller was used to apply stable and unidirectional fluid shear stress. Cell culture slides containing a confluent monolayer of HUVECs were placed in a parallel-plate flow chamber and subjected to LSS (2dyne/cm^2) at 37°C, with the flow medium maintained in a 95% air /5%CO_2 mixture.

2.5. Reagents

Primary antibodies against p-Akt(Ser473) (CST #4060 1:1000), Akt (CST #4691 1:1000), p-IRF3(Ser396) (CST #83611 1:1000), p-IRF3(Ser386)(CST #37829 1:1000), IRF3(CST #4302 1:1000), ICAM1 (CST #67836 1:1000), and GAPDH (Servicebio GB11002 1:1000)were used.

2.6. Western blotting analysis

After treatment with LSS, HUVECs were discarded from the original medium and washed with cold phosphate buffer (PBS). RIPA buffer containing protease inhibitors and phosphatase inhibitors was dissolved on ice at 4°C and centrifuged at 12000 RPM for 20 min before the supernatant was collected. Lysates were diluted with SDS (5×) and boiled for 10 min. Isolation of 20 μg of protein from pre-stained protein markers using 10% SDS-PAGE gel (THERMO, #26616). After transfer to a PVDF membrane (Bio-Rad, USA), the membrane was sealed in TBST buffer containing 3% bovine serum albumin for 2 h and

incubated with the primary antibody overnight at 4°C. The membrane was washed in TBST and incubated with the corresponding secondary antibody at room temperature for 1h. Immune responses that could be detected by chemiluminescence were detected using the ECL Plus assay kit, and protein bands were quantified using NIH Image J software 1.43.

2.7. Quantitative real-time PCR

Total RNA was extracted from HUVECs with or without LSS using TRIzol reagent. Complementary DNA strands were synthesized using the HiScrip III First Strand cDNA Synthesis Kit (Cat#R312-01/02, Vazyme, China). Rt-qPCR was performed using ChamQ Universal SYBR qPCR Master Mix(Cat#Q711-02, Vazyme, China) in a 7500 real-time PCR system, and the primer sequences for the real-time PCR assays are listed as follows:

ICAM-1 Forward:5'-CACAGTCACCTATGGCAACG – 3'

Reverse:5'-TCCTTGATCTTCCGCTGGC – 3'

VCAM-1 Forward:5'-CCTTCATCCCTACCATT – 3'

Reverse:5'-GTGTTTGCGTACTCTGC – 3'

GAPDH Forward:5'-GGACCTGACCTGCCGTCTAG-3'

Reverse:5'-AGCCCAGGATGCCCTTGAG-3'

Target relative mRNA levels were analyzed using the $2^{-\Delta\Delta Ct}$ method.

2.8. Transfection

Wild-type Akt plasmid (1477 pcDNA3 flag HA Akt1) was available on the Addgene website. HUVECs were incubated with serum-free OPTI-MEM and transfected with the corresponding plasmid using Lipofectamine 3000 (Thermo Fisher Scientific, L3000001). After 48h of transfection, cells were stimulated with low shear stress and collected for further experiments.

2.9. Statistical analysis

Values are presented as the mean ± S.E.M. from at least three independent experiments and were analyzed using GraphPad Prism 5 Software (GraphPad Software, Inc., San Diego,CA, USA). Statistical evaluation was performed using the Student's t-test for unpaired data and one-way analysis of variance. Statistical significance was set at $P < 0.05$.

3. Results

3.1 Berberine inhibits LSS-induced vascular endothelial inflammation.

To clarify the role of BBR in LSS-induced vascular endothelial inflammation, the effect of BBR on LSS-induced ICAM-1 expression was examined in vivo and in vitro. The results showed that compared with the control group, BBR treatment at 10μM concentration significantly inhibited ICAM-1 protein expression triggered by LSS at the cell level(Fig. 1A). Immunofluorescence analysis of ICAM-1 showed a similar trend (Fig. 1B). BBR also downregulated LSS-induced ICAM-1 and VCAM-1 mRNA expression (Fig. 1C). Similarly, BBR markedly downregulated intimal ICAM-1 expression in the aortic arch inner curvature (LSS) areas (Fig. 1D). These results suggest that BBR reverses LSS-induced endothelial inflammation.

3.2 Berberine inhibits LSS-induced Akt phosphorylation and IRF3 activation.

To explore the mechanism by which berberine inhibits endothelial inflammation and the effect of berberine on Akt/IRF3 activation, HUVECs were pretreated with BBR (5, 10, or 20 μM) for 24 h and then subjected to an LSS of 2 dyn/cm^2 for 60 min. Western blotting results showed that berberine decreased the phosphorylation level of Akt at Ser473 induced by LSS. The results showed that Akt phosphorylation was significantly inhibited at a dosage of 5μM. However, when the berberine dosage was increased to 10μM and 20μM, the phosphorylation of Akt did not decrease significantly compared with 5μM group, indicating that berberine did not inhibit Akt activation in a dose-dependent manner within the dose range of 5μM-20μM. In this study, a dosage of 10μM was used to explore this mechanism(Fig. 2A). We further examined the effects of BBR on IRF3 expression. Western blotting and immunofluorescence showed that BBR significantly eliminated LSS-induced IRF3 phosphorylation and nuclear translocation compared to the control (Fig. 2B-C). Similarly, BBR markedly downregulated intimal IRF3 expression in the aortic arch inner curvature (LSS) areas (Fig. 2D).

3.3 The inhibitory effect of berberine on LSS-induced IRF3 activation and ICAM-1 expression depends on Akt.

To investigate whether the inhibitory effect of berberine on LSS-induced ICAM-1 expression depends on Akt, HUVECs was overexpressed Akt1 by transfection with Akt1-WT plasmids, followed by berberine pre-treatment and LSS exposure. Western blotting was used to detect the effect of Akt1 overexpression. Protein bands indicated that the Akt1 plasmid was successfully transfected(Fig. 3A). However, in HUVECs transfected with Akt1-WT plasmids, LSS-elevated ICAM-1 expression and IRF3 phosphorylation could not be abolished by berberine treatment(Fig. 3B), as well as the LSS-induced nuclear translocation of IRF3 and ICAM-1expression assessed by immunofluorescence assay (Fig. 3C-D). In the LSS segments of the partially ligated common carotid artery, berberine markedly reduced intimal ICAM-1 expression in groups injected with control vectors. Nevertheless, the inhibitory effect of berberine on LSS-induced ICAM-1 expression vanished in EC-specific Akt1 overexpression groups with pAAV9-TIE-Akt1 vector injection(Fig. 4A-B). These results indicate that the inhibitory effect of berberine on LSS-induced IRF3 activation and ICAM-1 expression relies on Akt.

4. Discussion

Although previous studies have suggested that berberine has potential anti-arteriosclerotic effects, its role and mechanism of action in LSS-induced endothelial inflammation remain unclear. Our study demonstrated that berberine treatment attenuated LSS-induced vascular endothelial inflammation by decreasing activation of the Akt/IRF3 signalling pathway, indicating a protective role of berberine in LSS-induced endothelial inflammation.

Berberine (BBR), a proto-berberine alkaloid, is a naturally occurring compound with a wide range of pharmacological activities[15]. Recently, berberine was reported to alleviate atherosclerosis by reducing oxidative stress and vascular inflammation via the stimulation of AMPK-dependent UCP2 expression[16]. In addition, berberine inhibited MMP-1 and vascular inflammation induced by glutamate in newborn rats[17]. Berberine also protects HUVECs from oxLDL-induced ROS production and subsequent cellular dysfunction[18]. Although our previous study found that berberine attenuated LSS-induced hyaluronic acid degradation by increasing AMPKα phosphorylation [14], the role of berberine in LSS-induced endothelial inflammation remains unknown. In the present study, both in vitro and in vivo experiments showed that berberine reduced the LSS-induced endothelial expression of ICAM-1 and VCAM-1, suggesting a protective role of berberine against LSS-induced endothelial inflammation. The novel finding that berberine inhibits LSS-induced endothelial inflammation indicates that berberine may be an effective treatment for mitigating LSS-induced arteriosclerosis.

Our previous study revealed the role of the Akt/IRF3 signalling pathway in LSS-induced vascular endothelial inflammation. Briefly, LSS-induced Akt activation is mediated by IKKε and leads to vascular endothelial inflammation by promoting IRF3 phosphorylation and its nuclear translocation[11]. However, effective interventions for this pathological mechanism are lacking. Emerging studies have revealed the effects of berberine on the Akt/IRF3 signalling pathway. Berberin improves diabetes-related retinal endothelial dysfunction by inhibiting Akt/mTOR-mediated HIF-1α/VEGF activation[13]. Moreover, berberine reduced carotid atherosclerosis by modulating the PI3K/AKT/mTOR signaling pathway. Studies concerning the effect of berberine on IRF3 are rare, and existing research has shown that berberine inhibits the phosphorylation of IRF3 and the inflammatory response of macrophages in an LPS-induced mouse sepsis model[19]. To date, the effect of berberine on LSS-induced Akt/IRF3 activation remains unclear.

In the present study, we demonstrated that berberine significantly inhibited LSS-induced Akt phosphorylation as well as IRF3 activation in HUVECs and mouse aortic arch inner curvature. However, Akt1 overexpression abolished the inhibitory effect of berberine on LSS-induced IRF3 activation and ICAM-1 expression, indicating that Akt is key for berberine to suppress LSS-induced inflammation.

With HUVECs and berberine-treated mouse model, our results demonstrate that berberine ameliorates LSS-mediated endothelial inflammation through reducing the activation of Akt and IRF3 (Fig. 5). However, there are still some limitations to our study. First, although our study showed that berberine inhibited LSS-induced vascular endothelial inflammation, its role in LSS-induced atherosclerosis has not been investigated. Second, although berberine has been clinically used in the treatment of intestinal infections,

there is still no evidence for its application in cardiovascular diseases, and new derivatives need to be developed to improve its bioavailability and clinical efficacy.

Declarations

Author contributions

Linlin Zhu and Shaoliang Chen conceptualized and designed the research, Yifei Lv, Hongfeng Yang and Peng Ye performed the experiments, Zhiyuan Qian,Dongchen Wang, Chaohua Kong, Yunfei Deng, Yue Gu and Wenying Zhou analyzed and interpreted experimental results. Yifei Lv drafted the manuscript.

Conflict of Interest Statement

No conflicts of interest, financial or otherwise, are declared by the authors.

Acknowledgments

This study was supported by grants from the Natural Science Foundation of Jiangsu Province (BK20191117).

Data availability

All data generated or analyzed during this study are included in this published article.

Ethics approval

The animal experiments were approved by the Ethics of Animal Experiments of Nanjing Medical University, China.

References

1. Taleb S (2016) Inflammation in atherosclerosis. Arch Cardiovasc Dis 109:708–715. doi: 10.1016/j.acvd.2016.04.002
2. Wang XL, Fu A, Raghavakaimal S and Lee HC (2007) Proteomic analysis of vascular endothelial cells in response to laminar shear stress. Proteomics 7:588–596. doi: 10.1002/pmic.200600568
3. Burghoff S and Schrader J (2011) Secretome of human endothelial cells under shear stress. J Proteome Res 10:1160–9. doi: 10.1021/pr100937a
4. Bondareva O, Tsaryk R, Bojovic V, Odenthal-Schnittler M, Siekmann AF and Schnittler HJ (2019) Identification of atheroprone shear stress responsive regulatory elements in endothelial cells. Cardiovasc Res 115:1487–1499. doi: 10.1093/cvr/cvz027
5. Mohan S, Koyoma K, Thangasamy A, Nakano H, Glickman RD and Mohan N (2007) Low shear stress preferentially enhances IKK activity through selective sources of ROS for persistent activation of NF-kappaB in endothelial cells. Am J Physiol Cell Physiol 292:C362-71. doi: 10.1152/ajpcell.00535.2005

6. Tsou JK, Gower RM, Ting HJ, Schaff UY, Insana MF, Passerini AG and Simon SI (2008) Spatial regulation of inflammation by human aortic endothelial cells in a linear gradient of shear stress. Microcirculation 15:311–23. doi: 10.1080/10739680701724359
7. Chao Y, Zhu L, Qu X, Zhang J, Zhang J, Kong X, Gu Y, Pu J, Wu W, Ye P, Luo J, Yang H and Chen S (2017) Inhibition of angiotension II type 1 receptor reduced human endothelial inflammation induced by low shear stress. Exp Cell Res 360:94–104. doi: 10.1016/j.yexcr.2017.08.030
8. Tsuchiya K, Tanaka J, Shuiqing Y, Welch CL, DePinho RA, Tabas I, Tall AR, Goldberg IJ and Accili D (2012) FoxOs integrate pleiotropic actions of insulin in vascular endothelium to protect mice from atherosclerosis. Cell Metab 15:372–81. doi: 10.1016/j.cmet.2012.01.018
9. Dimmeler S, Assmus B, Hermann C, Haendeler J and Zeiher AM (1998) Fluid shear stress stimulates phosphorylation of Akt in human endothelial cells: involvement in suppression of apoptosis. Circ Res 83:334–41. doi: 10.1161/01.res.83.3.334
10. Lee HJ and Koh GY (2003) Shear stress activates Tie2 receptor tyrosine kinase in human endothelial cells. Biochem Biophys Res Commun 304:399–404. doi: 10.1016/s0006-291x(03)00592-8
11. Zhu L, Yang H, Chao Y, Gu Y, Zhang J, Wang F, Yu W, Ye P, Chu P, Kong X and Chen S (2021) Akt phosphorylation regulated by IKKepsilon in response to low shear stress leads to endothelial inflammation via activating IRF3. Cell Signal 80:109900. doi: 10.1016/j.cellsig.2020.109900
12. Rui R, Yang H, Liu Y, Zhou Y, Xu X, Li C and Liu S (2021) Effects of Berberine on Atherosclerosis. Front Pharmacol 12:764175. doi: 10.3389/fphar.2021.764175
13. Wang N, Zhang C, Xu Y, Tan HY, Chen H and Feng Y (2021) Berberine improves insulin-induced diabetic retinopathy through exclusively suppressing Akt/mTOR-mediated HIF-1alpha/VEGF activation in retina endothelial cells. Int J Biol Sci 17:4316–4326. doi: 10.7150/ijbs.62868
14. Yang H, Zhu L, Gu Y, Kong X, Yan L, Chen M, Xie X, Luo J and Chen S (2019) Berberine inhibits low shear stress-induced glycocalyx degradation via modulating AMPK and p47(phox)/Hyal2 signal pathway. Eur J Pharmacol 856:172413. doi: 10.1016/j.ejphar.2019.172413
15. Chen CC, Hung TH, Lee CY, Wang LF, Wu CH, Ke CH and Chen SF (2014) Berberine protects against neuronal damage via suppression of glia-mediated inflammation in traumatic brain injury. PLoS One 9:e115694. doi: 10.1371/journal.pone.0115694
16. Wang Q, Zhang M, Liang B, Shirwany N, Zhu Y and Zou MH (2011) Activation of AMP-activated protein kinase is required for berberine-induced reduction of atherosclerosis in mice: the role of uncoupling protein 2. PLoS One 6:e25436. doi: 10.1371/journal.pone.0025436
17. Abo Zeid AA, Rowida Raafat I and Ahmed AG (2020) Berberine alleviates monosodium glutamate induced postnatal metabolic disorders associated vascular endothelial dysfunction in newborn rats: possible role of matrix metalloproteinase-1. Arch Physiol Biochem:1–12. doi: 10.1080/13813455.2020.1729815
18. Hsieh YS, Kuo WH, Lin TW, Chang HR, Lin TH, Chen PN and Chu SC (2007) Protective effects of berberine against low-density lipoprotein (LDL) oxidation and oxidized LDL-induced cytotoxicity on endothelial cells. J Agric Food Chem 55:10437–45. doi: 10.1021/jf071868c

19. Li H, Wang Y, Zhang H, Jia B, Wang D, Li H, Lu D, Qi R, Yan Y and Wang H (2012) Yohimbine enhances protection of berberine against LPS-induced mouse lethality through multiple mechanisms. PLoS One 7:e52863. doi: 10.1371/journal.pone.0052863

Figures

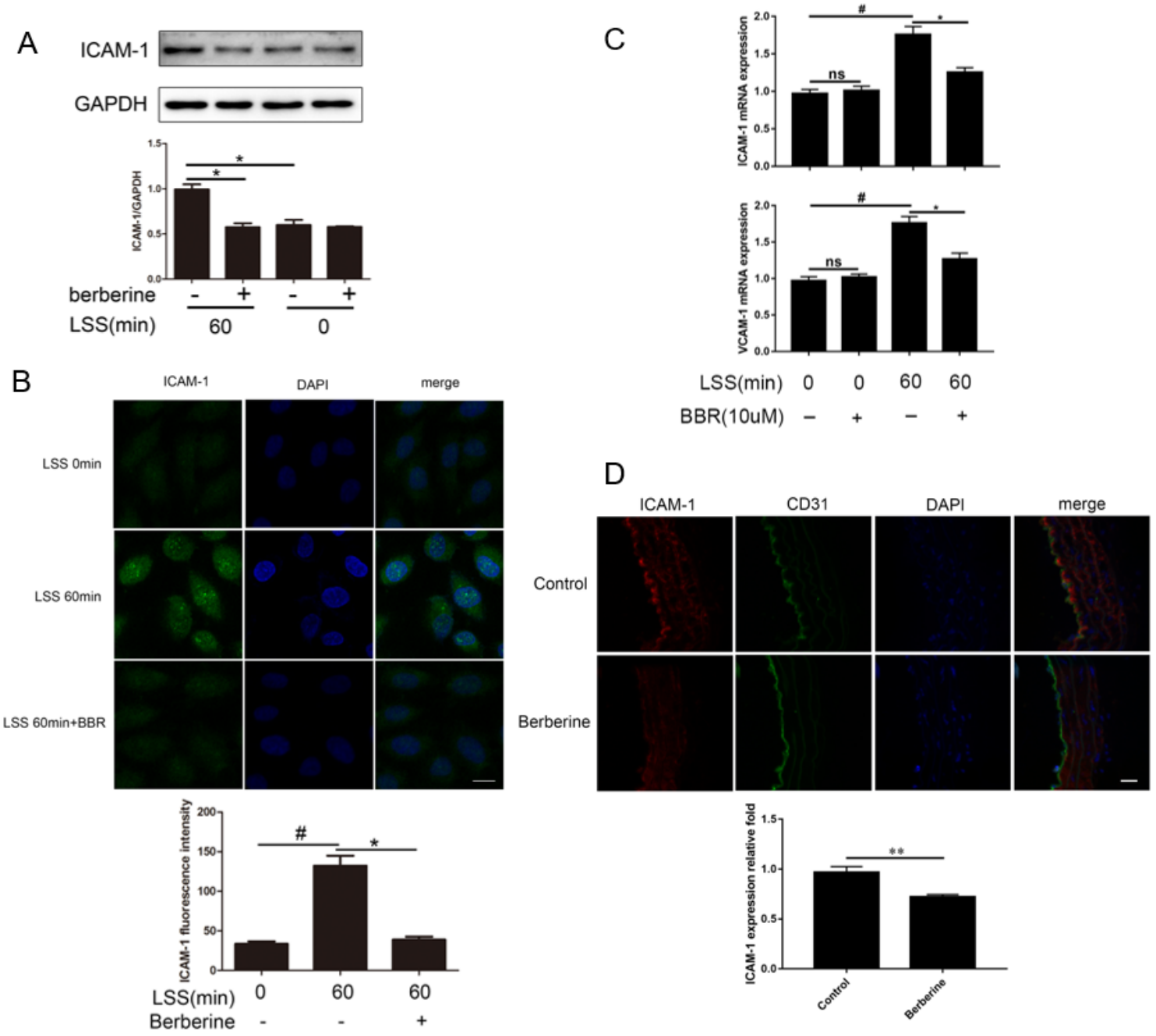

Figure 1

Berberine inhibits LSS-induced endothelial inflammation in vitro. (A). HUVECs were pretreated with BBR (10μM) or DMSO 1:1000 for 24h and then subjected to low shear stress stimulation for 60min.ICAM-1 protein expression were determined by Western blot(n=3). (B). HUVECs were exposed to LSS with or

without pretreatment with BBR. ICAM-1expressions were analyzed by immunofluorescence. (green). Nuclei were stained by DAPI (blue) (n=3). Scale bar=20μm. (C). HUVECs were pretreated with BBR (10μM) or DMSO 1:1000 for 24h and then subjected to low shear force stimulation for 60min. The relative ICAM-1 and VCAM-1 mRNA were detected by RT-qPCR (n=3). (D). The intimal ICAM-1 expression of the aorta arch inner curvature in mice treated with or without BBR(n=6). Scale bar = 50μm.Data are presented as mean ± SEM. *$p < 0.05$ vs. 60min, #$p < 0.05$ vs. static condition. **$p < 0.05$ vs. the normal saline group.

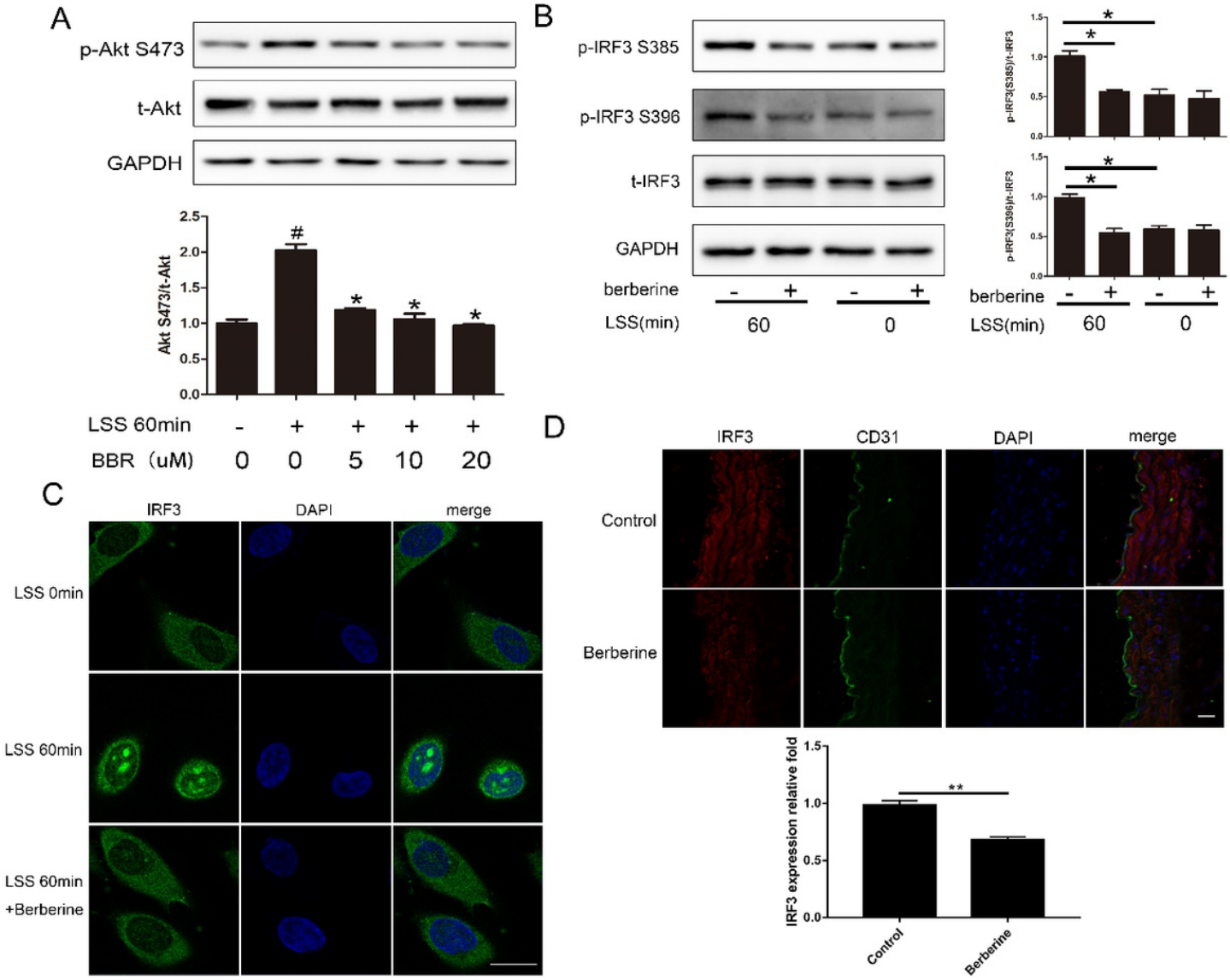

Figure 2

Berberine inhibits LSS-induced Akt phosphorylation and IRF3 activation. (A). HUVECs were pretreated with BBR(5, 10, and 20 μM) for 24h and then expossed to low shear stress of 2 dyn/cm^2 for 60min. Akt phosphorylation was measured using western blot(n=3). (B). HUVECs were pretreated with or without berberine (10μM) and then exposed to low shear stress of 2 dyn/cm^2 for 60min. The phosphorylation of IRF3 was measured using western blot(n=3). (C). HUVECs were exposed to LSS with or without berberine pretreatment. IRF3 location were measured by immunofluorescence. IRF3 was recognized with anti-IRF3

antibody and incubated with FITC-conjugated secondary antibody (green). DAPI stained nucleus (blue) (n=3). Scale bar=20μm. (D). The intimal IRF3 expression of the aorta arch inner curvature in mice treated with or without berberine(n=6). Scale bar=50μm. Data are presented as mean ± SEM. *$p < 0.05$ vs. 60min, #$p < 0.05$ vs. static condition. **$p < 0.05$ vs.the normal saline group.

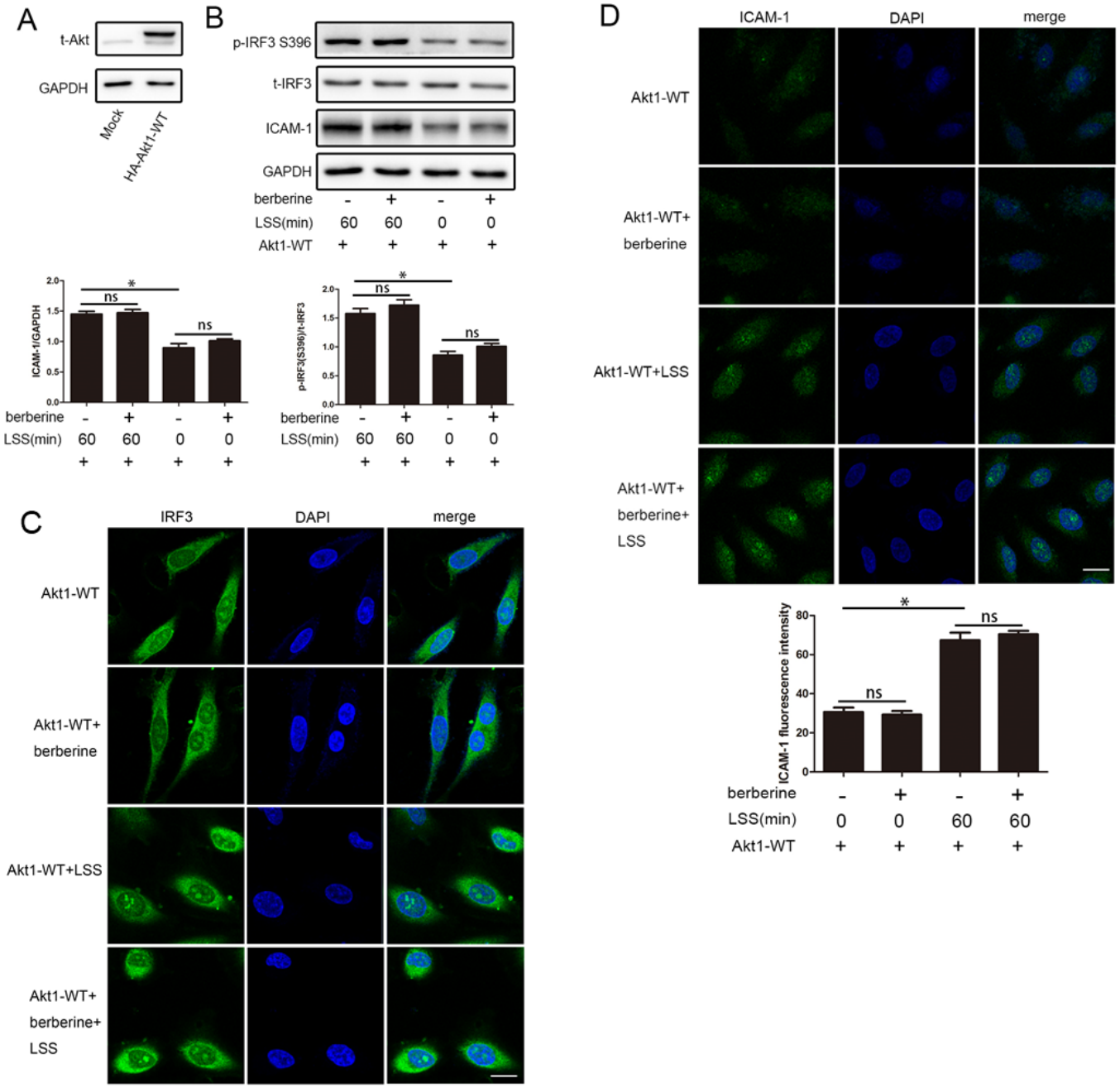

Figure 3

The inhibitory effect of berberine on LSS-induced IRF3 activation and ICAM-1 expression depends on Akt. (A,B). After being transfected with Akt1-WT plasmids, HUVECs were pretreated with or without BBR and then exposed to LSS. ICAM-1 expression and IRF3 phosphorylation were assessed by Western blot(n=3). (C,D). After being transfected with Akt1-WT plasmids, HUVECs were pretreated with or without BBR and

then exposed to LSS. IRF3 location and ICAM-1 expression were analyzed by immunofluorescence (n=3). Scale bar=20μm. Data are presented as mean ± SEM. *$p < 0.05$ vs. 60min.

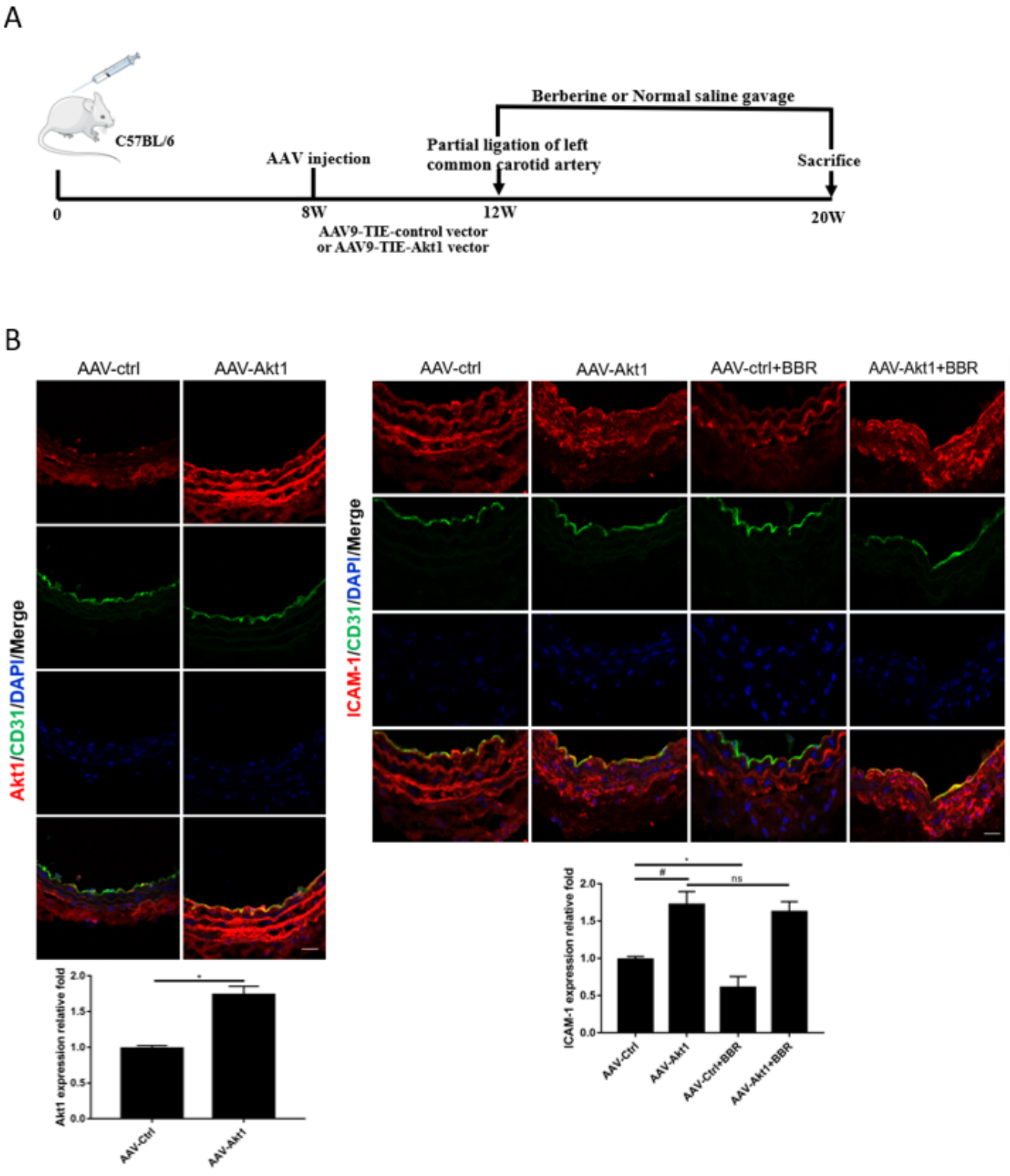

Figure 4

The inhibitory effect of berberine on LSS-induced ICAM-1 expression depends on Akt in C57BL/6 mice. (A). C57BL/6 mice were injected with AAV-TIE-Akt1 vector or AAV9-TIE-control vector via tail vein. Four

weeks later, partially ligated left common carotid arteries and the mice were gavaged with berberine or normal saline for 8 weeks. (B). After the ligation of the left common carotid arteryl, the expression levels of Akt1 and ICAM-1 in intima of the low shear stress region were detected by immunofluorescence assay. (n=6).Scale bar=50μm. Data are presented as mean ± SEM. *$p < 0.05$ vs. the AAV-ctrl group. #$p < 0.05$ vs. the AAV-ctrl group.

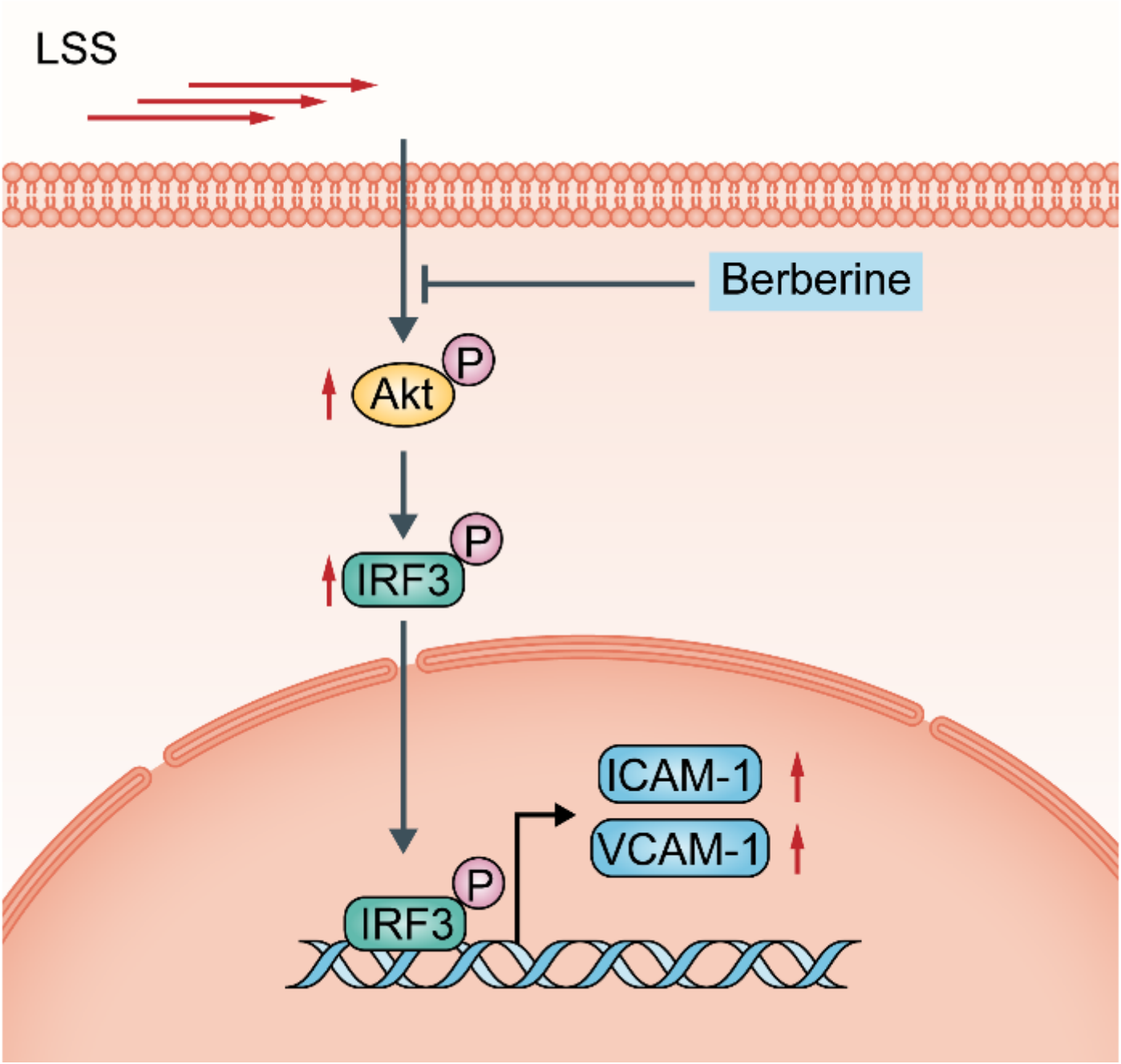

Figure 5

Schematic diagram of berberine's role in reversing LSS-induced vascular endothelial inflammation.

Berberine reversed LSS-induced vascular endothelial inflammation by decreasing Akt activation, thereby inhibiting IRF3 phosphorylation and nuclear translocation.