

Berberine Exhibits Neuroprotective Effects Through Reduced Autophagy and Mediated Microglial Polarization in a Rat Model of Intracerebral Hemorrhage

Qiang Lei

department of neurology, the second xiangya hospital, central south university, changsha, hunan

Zhiping Hu

Second Xiangya Hospital, the second xiangya hospital, central south university, changsha, Hunan

Binbin Yang

department of neurology, the second xiang hospital, central south university, changsha, hunan

Fangfang Zhou (✉ zhoufangfang6822@csu.edu.cn)

The Second Xiangya Hospital, Central South University

Research

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Abstract

Introduction: Intracerebral haemorrhage (ICH) induces autophagy excessive activation and microglia mainly switched into proinflammatory M1 type, which can cause severe secondary injury. Current evidence has implied that berberine has a protective effect against ischaemic stroke through mediated autophagy and microglial polarization. However, the neuroprotective effect of berberine in intracerebral haemorrhage (ICH) remains unclear.

Methods: In this study the effect of berberine on a rat model of intracerebral haemorrhage was investigated through Immunofluorescence, qPCR, ELISA, and western blot.

Results: Berberine administration significantly reduces cellular apoptosis, brain edema and neurological deficits via inhibited autophagy, promoted M1 type microglia to M2 type, and subsequently exerts anti-inflammation effects in a rat model of ICH.

Conclusions: These results suggest that berberine reduced secondary injury and improved neurological outcomes in ICH model.

Introduction

Intracerebral haemorrhage (ICH) was a devastating type of stroke with approximately 40% mortality at one month and high disability after hospital. Mass effect from hematoma induced the primary brain damage, while secondary brain injury, including toxic effects of hemolytic products, oxidative stress and inflammatory activation, was an important factor that led to neurological outcome progress[1]. Currently, Effective treatment in ICH is limited, hence it is urgently to carry out related studies.

Autophagy is self-degradation of damaged organelles and misfolded or injured protein in order to maintain cellular hemostasis. Autophagy participated in many pathophysiological processes, including cell survival, differentiation, proliferation and inflammation[2]. A variety of studies focus on the role of autophagy in neurological disease. Research presented that promoting autophagy had protective effect in animal models of subarachnoid hemorrhage[3], traumatic brain injury[4] and Parkinson diseases[5], while the effect of promoting autophagy is controversial in ischemic stroke. However, recent studies presented that excessive autophagy occurs after ICH which possibly contributes to brain injury and cell apoptosis.

Microglia were cerebral resident macrophages and were originated from myeloid progenitor cells. Microglia were activated into two phenotypes: M1 microglia was pro-inflammation and M2 microglia is anti-inflammation. In the early phase of ICH, microglia were activated and mainly switched into M1 microglia polarization. M1 type released proinflammatory cytokines and chemokines, which lead to secondary brain injury[6]. Studies demonstrated that inhibition of autophagy could switched M1 microglia to M2 phenotype in ischemic stroke, which exerted anti-apoptosis effect[7].

Berberine is a nature alkaloid collected from herb *Coptis chinensis*, which is widely used as an anti-inflammatory, antidiabetic and antihyperlipidemic drug. Berberine exert protective effect in ischemic stroke via mediating autophagy and microglia polarization[8, 9]. Dent et al found that berberine could inhibit autophagy through downregulating the expression of BECN1[10]. Previous studies presented that inhibition of autophagy ameliorated neurological outcome in ICH[11]. However, the role of Berberine in ICH remained unclear. Hence, we initially explored that berberine possibly exerted neuroprotection via autophagy inhibited and promoting M1-to-M2 microglia phenotype.

Methods

Animals and groups

The protocols of animal usage were approved by the Institutional Animal care and Use Committee of the Second Xiangya hospital of Central South University in compliance with NIH guidelines. Sprague-Dawley adult male rats with 250 to 300g were employed in our study. Animals were kept at a constant temperature and a 12/12h light/dark cycle. Rats (n = 54, 2 sacrificed) is randomized divided into three groups: (1) ICH + Berberine (Beri group, n = 17), rats were administered with berberine intragastrically (sigma, 50mg/kg) daily for 10 consecutive days before ICH and 3 days after ICH; (2) ICH + vehicle (vehicle group, n = 17); (3) sham-operated group (sham group, n = 18).

ICH models

Rats were anaesthetized with chloral hydrate and placed on a stereotaxic frame. 0.2U in 2.0 μ L collagenase type IV was injected into the right hemisphere at 3.0 mm lateral to the midline, 0.2 mm anterior to bregma and 6 mm deep by micro pump with 5 minutes. The needle was maintained in brain for 5 minutes after injection. The sham group carried out the same procedures with no collagenase type IV injection.

Behaviour analysis

Neurological deficit scores were evaluated by modified neurological severity score (mNSS, n = 8) 24 and 72 hours after ICH. The mNSS is an 18-point scale and maximal deficit is 18. Rats from each group were assessment.

Terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) staining

Perihematoma paraffin section (4 μ m, n = 4) was dewaxed with xylene and ethyl alcohol. Apoptosis was examined with a TUNEL staining kit (Roche, Swit) according to the manufacture's instructions. The slide was examined with a fluorescence microscope (Olympus, Japan).

Immunofluorescence

Perihematomal paraffin section (4µm, n = 4) was dewaxed with xylene and ethyl alcohol. Brain section was antigenically repaired with EDTA buffer, added with spontaneous fluorescence quenching reagent and serum. Primary and secondary antibody were added. Antibody were included LC3B (Proteintech Inc, US, 18725-1-AP). Cell nucleus was stained with DAPI. Immunofluorescence was observed with a fluorescence microscope (Olympus, Japan).

Enzyme-linked immunosorbent assay (ELISA)

Perihematomal cerebral tissue (sham group n = 5, vehicle and Beri group n = 4) was obtained 72 hours after ICH. The levels of TNF-α, IL-1β, IL-6, IL-10 were examined by a commercial ELISA kit (Cusabio, China) according to the manufacturer's instructions.

qPCR

Total RNA (n = 5) was collected from perihematomal brain tissue 72 hours after ICH. The specific primer was used to synthesize the complementary DNA. PCR conditions were performed according to the manufacturer's instructions. The analysis of qPCR was used by a PCR instrument. Data was normalized to actin. mRNA expression was relative to sham group. The primer sequences were as follows: iNOS (forward, 5'- AGGCACAAGACTCTGACACCC-3', reverse, 5'- CGCACTTCTGTCTCTCCAAA

CCC - 3'), CD32 (forward, 5'- TCAATCCCCAAAGCCAACCAC - 3', reverse, 5'- ATAACAATGGCAGCTACAGCAA-3'), CD206 (forward, 5'- TATCTCTC

CAACCACGGCACCA - 3', reverse, 5'- AGTATTTCTCTGCTTCGTGCCAT - 3'), Arg1 (forward, 5'- CATATCTGCCAAGGACATCGT - 3', reverse, 5'- TCCAT

CACTTTGCCAATTCCC - 3'). RT-qPCR analysis was undergone with a Bio-Rad iCycler using their SYBR Green PCR mix (Bio-Rad, USA). RT-qPCR conditions were performed as the manufacturer's instructions. Data were normalized to β-actin.

Western blot

Protein (n = 4) was obtained from perihematomal tissue 72 hours after ICH. Protein were separated by SDA-PAGE electrophoresis, transferred to PVDF membranes and then incubated with primary antibodies. Primary antibodies included LC3 (Proteintech Inc, US, 14600-1-AP) and Beclin 1 (Proteintech Inc, US, 11306-1-AP). Densities were analysis with ImageJ software.

Statistical Analysis

GraphPad Prism 5.01 software was used for statistical analyses. All data were presented as the mean ± standard deviation (mean ± SD). Comparisons between two groups were analyzed with Mann-Whitney test or ANOVA followed by Bonferroni test. Statistical significance was set as P<0.05.

Results

Berberine reduced mNSS scores and apoptosis in ICH

This study found that Beri group had significantly lowered mNSS scores (Fig. 1A) at 72 hours after ICH compared with the vehicle group. Meanwhile, Berberine obviously reduced cerebral edema 72 hours after ICH (Fig. 1B). Additionally, berberine markedly reduced apoptotic cells in the perihematoma (Fig. 2) which was analysis with TUNEL staining. These were suggested that berberine exerted obviously protective effects in ICH.

Berberine declined excessively activated autophagy in ICH

LCII and beclin-1 are two markers to determine the activation of autophagy. The LC3II staining was significantly increased in perihematoma area after ICH (Fig. 3A). The LC3-II/LC3-I ratio and expression of beclin-1 were markedly augmented after ICH (Fig. 3B). These results indicated that autophagy was evidently activated in perihematoma area after ICH. Beri group show significantly lower LC3II staining, LC3-II/LC3-I ratio and expression of beclin-1 compared with vehicle group (Fig. 3). These results implied that berberine significantly reduced autophagy compared with vehicle in ICH.

Berberine promoted M1 microglia switched into M2 microglia after ICH

The makers of microglia M1 and M2 were determined by qPCR, expressions of M1-related markers including CD32 and iNOS was obviously reduced in Beri groups while M2-related markers including CD206 and Arg1 was significantly increased in Beri groups compared with Vehicle group (Fig. 4). Meanwhile, levels of M1-related cytokines including IL-6, IL-1 β and TNF- α , investigated by Elisa, were significantly reduced after berberine treatment in ICH, while M2-related cytokines, the levels of IL-10, were significantly increased in Beri group compared with vehicle group (Fig. 5). Therefore, these indicated that berberine significantly facilitated M1 microglia transformed into M2 microglia, which could exert anti-inflammation effect after ICH.

Discussion

Previous studies demonstrated that berberine could reduce apoptosis in neurological diseases. Liang et al stated that berberine exhibited neuroprotective effect via against amyloid β -protein induced apoptosis[12]. Studies presented that berberine could improve brain traumatic brain injury against neuronal damage via anti-inflammation and Sirt1/P38 signaling[13]. Meanwhile, berberine inhibited nigrostriatal dopaminergic neuronal loss and protected against hippocampal apoptosis in mice model of Parkinson's disease[14]. Berberine significantly attenuated neuronal apoptosis in status epilepticus[15]. Additionally, berberine could significantly reduce cell apoptosis after ischemic stroke via phosphoinositide 3-kinase signaling pathway[16]. Simultaneously, our study initially found that berberine could decrease cell apoptosis in perihematoma area after ICH, eventually improved neurological outcome.

Berberine ameliorated variety of diseases through mediating the autophagy[17]. Previous study presented that berberine decreased NLRP3 inflammasome activation by promoting autophagy in macrophages[18]. Jin et al found that berberine alleviated high glucose-induced podocytes injury through enhancing autophagy[19]. However, it is reported that berberine could protect kidney from cisplatin-induced damage through inhibition of autophagy[20]. Berberine significantly protected nucleus pulposus cell from oxidative stress damaged through reducing autophagy[21]. Jia et al found berberine improved hypoxia-induced myocytes damage through attenuating excessive autophagy[22]. Additionally, berberine exerted protective effects in ischemic stroke through alleviating autophagy[9]. In this study, we found that berberine play neuroprotective role in ICH by inhibiting autophagy.

Numerous studies found that berberine play a role in microglia activation and polarization[8]. Studies showed that berberine reduced amyloid- β -induced microglia activation and exerted anti-neuroinflammatory effects[23]. Berberine suppressed AMP-activated protein kinase induced microglia activation[24]. Meanwhile, berberine could suppress M1 microglia polarization and promote M2 microglia polarization in ischemic stroke[8]. Similarly, our study firstly showed that berberine enhanced M1-to-M2 microglia polarization and then inhibited neuroinflammation.

Autophagy was possibly associated with microglia polarization. Jin et al found that enhancement of autophagy significantly promoted M2 microglia polarization and inhibited neuroinflammation in Parkinson's diseases[25]. Augment of autophagy facilitated M1-to-M2 phenotypic shift in LPS-induced microglia[26]. Nevertheless, inhibition of autophagy markedly promoted M1 switch into M2 microglia polarization and reduced pro-inflammatory cytokines products in ischemic stroke[7]. In ischemic white matter damage, suppression of autophagy could accelerate M1-to-M2 microglia polarization[27]. Therefore, our study showed berberine promoted M1-to-M2 microglia transformation possibly through reduced autophagy.

Conclusions

Our study initially revealed that berberine treatment in ICH exerted neuroprotective effects through inhibited autophagy and promoted M1 transferred to M2 polarization, which significantly ameliorated neuroinflammatory injury. The study provides evidence that berberine is probably a novel therapy for ICH.

Abbreviations

ICH: intracerebral hemorrhage; Berberine: Beri; mNSS: modified neurological severity score; TUNEL: Terminal deoxynucleotidyl transferase dUTP nick end labelling; ELISA: Enzyme-linked immunosorbent assay; SD: standard deviation; Iba-1: ionized calcium binding adaptor molecule-1; IL-1 β : interleukin-1 β ; IL-6: interleukin-6; IL-10: interleukin-10; TNF- α : tumor necrosis factor- α ; iNOS: inducible nitric oxide synthase; Sirt1: Sirtuin 1; NLRP3: nucleotide-binding domain, leucine-rich containing family, pyrin-domain containing 3; LPS: Lipopolysaccharide

Declarations

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Availability of data and materials

Data generated or analyzed during this study are included in the published article.

Authors' contributions

QL and FFZ designed the research. QL, BBY and ZPH conducted the experiments, analyzed the data. QL and FFZ contributed to the production of the manuscript. All authors read and approved the final manuscript.

Ethics approval

All procedures were approved by the Second Xiangya Hospital, Central South University, Changsha, Hunan 410011, China.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflicts of interest.

Author details

1,2,3,4Department of neurology, The Second Xiangya Hospital, Central South University, Changsha, Hunan 410011, China.

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Figures

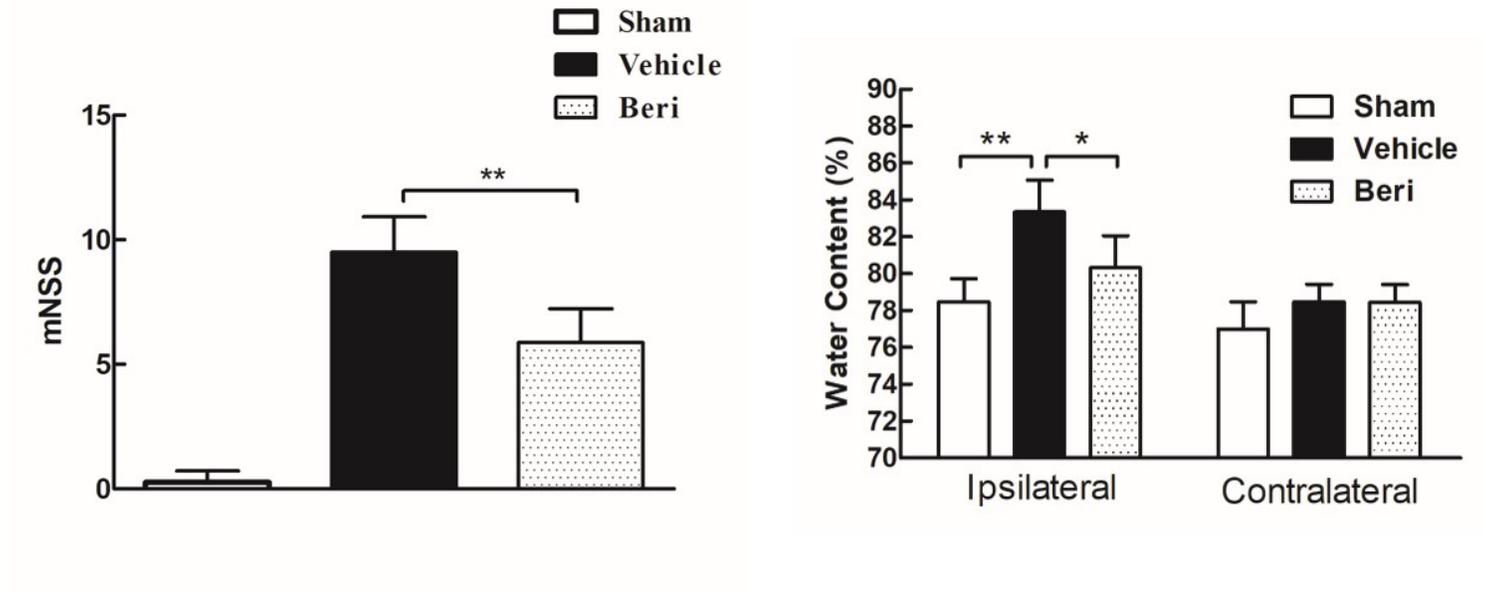


Figure 1

Treatment with Beri significantly reduced mNSS and brain water content of Ipsilateral cerebral. A. mNSS was evaluated 72 hours after ICH. B. Brain water content was examined 72 hours after ICH. Values are presented as the mean±SD; *p<0.05, **p<0.01 in all graphs.

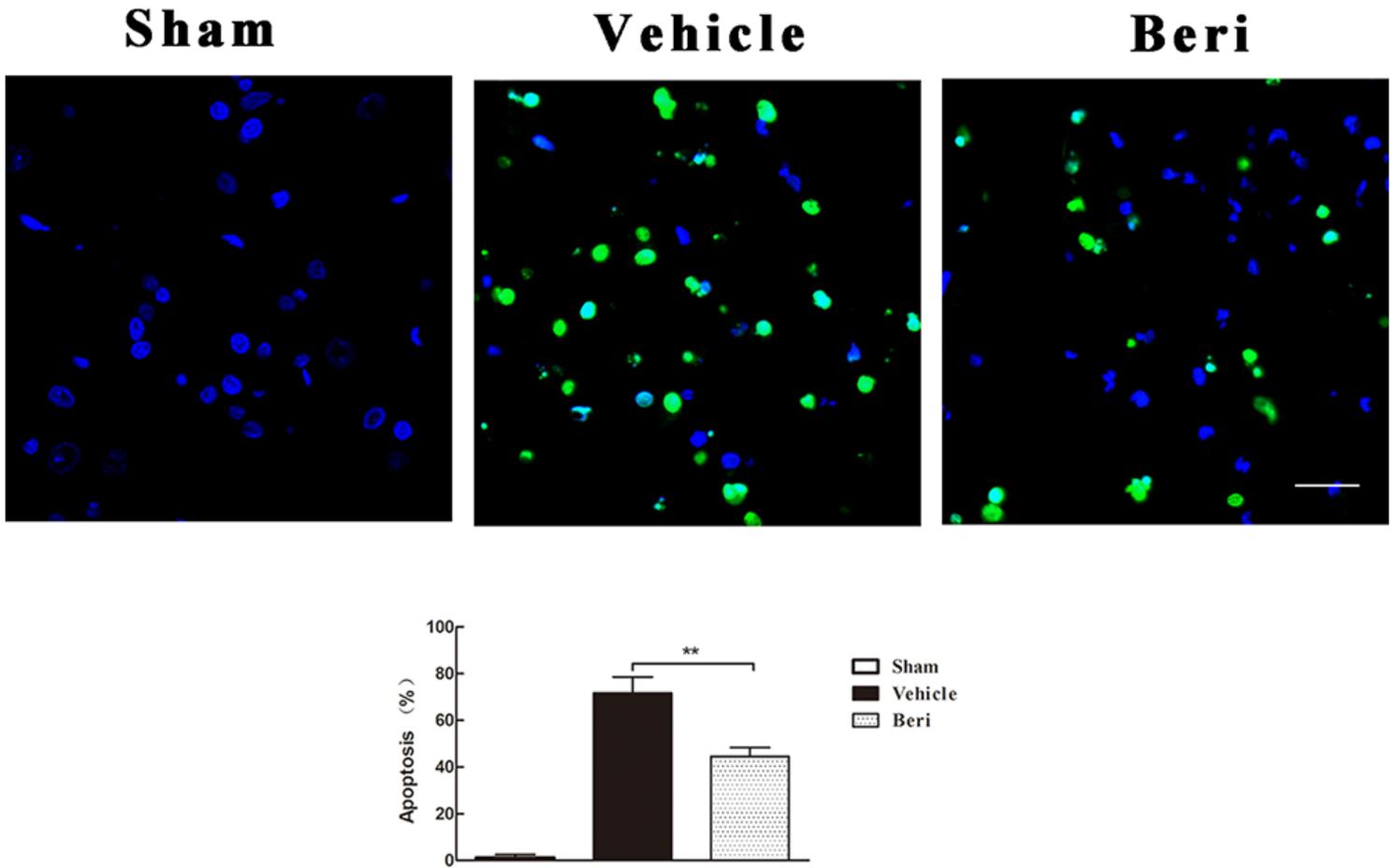


Figure 2

Treatment with Beri markedly attenuated apoptosis in the perihematomal area after ICH. Representative photographs showing immunostaining for TUNEL+ (Green) in the perihematomal area 72 hours after ICH (bar=20 μ m) and DAPI (blue). Values are presented as the mean \pm SD; *p < 0.05, **p < 0.01 in all graphs.

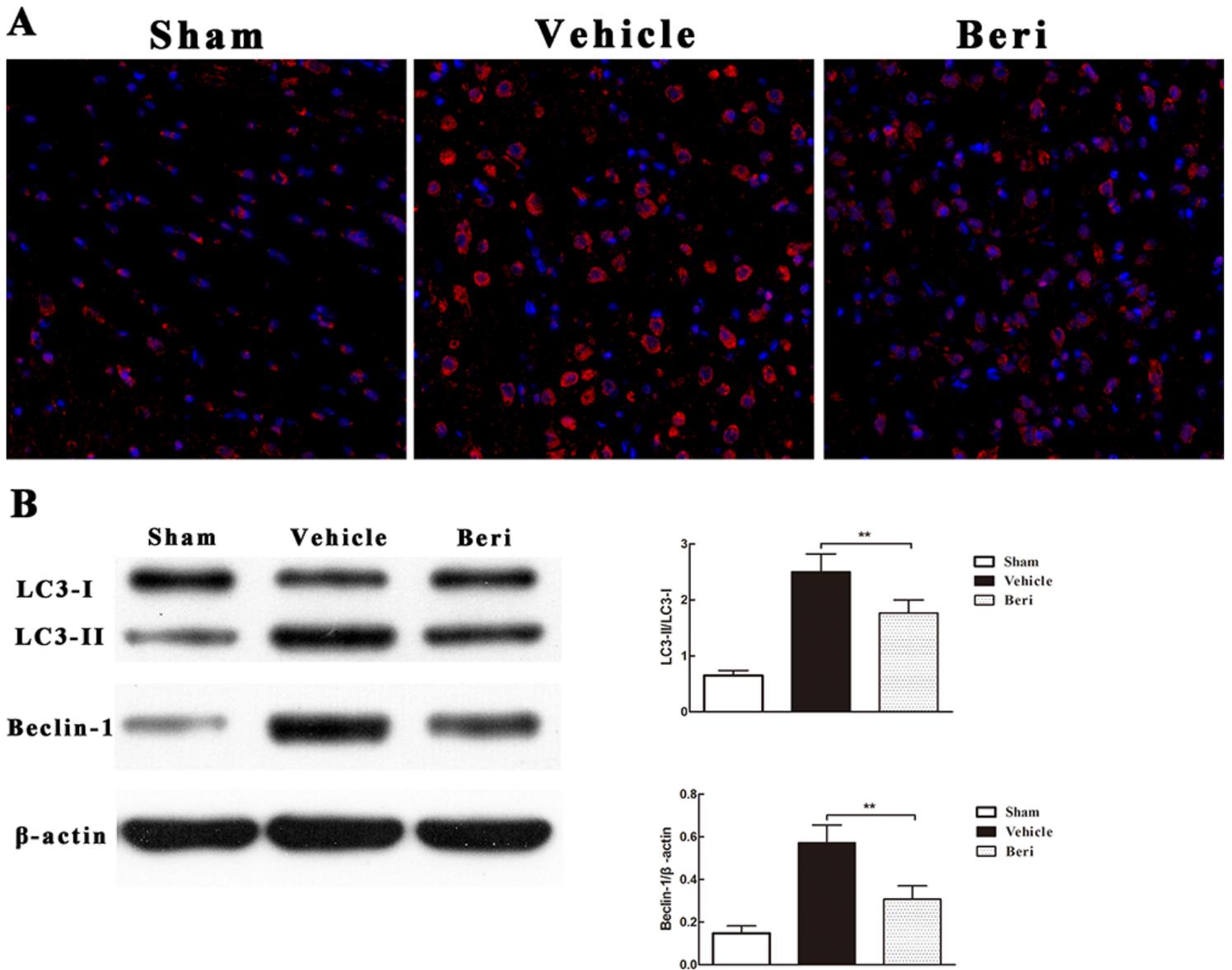


Figure 3

Treatment with Beri markedly suppressed the autophagy in the perihematomal area after ICH. A. Representative photographs showing immunostaining for LC3II(Red) in the perihematomal area 72 hours after ICH (bar=20 μ m) and DAPI (blue). B. Western blot showing the expression of LC3-II/LC3-I and Beclin-1 (bar=20 μ m). Values are presented as the mean \pm SD; * p <0.05, ** p <0.01 in all graphs.

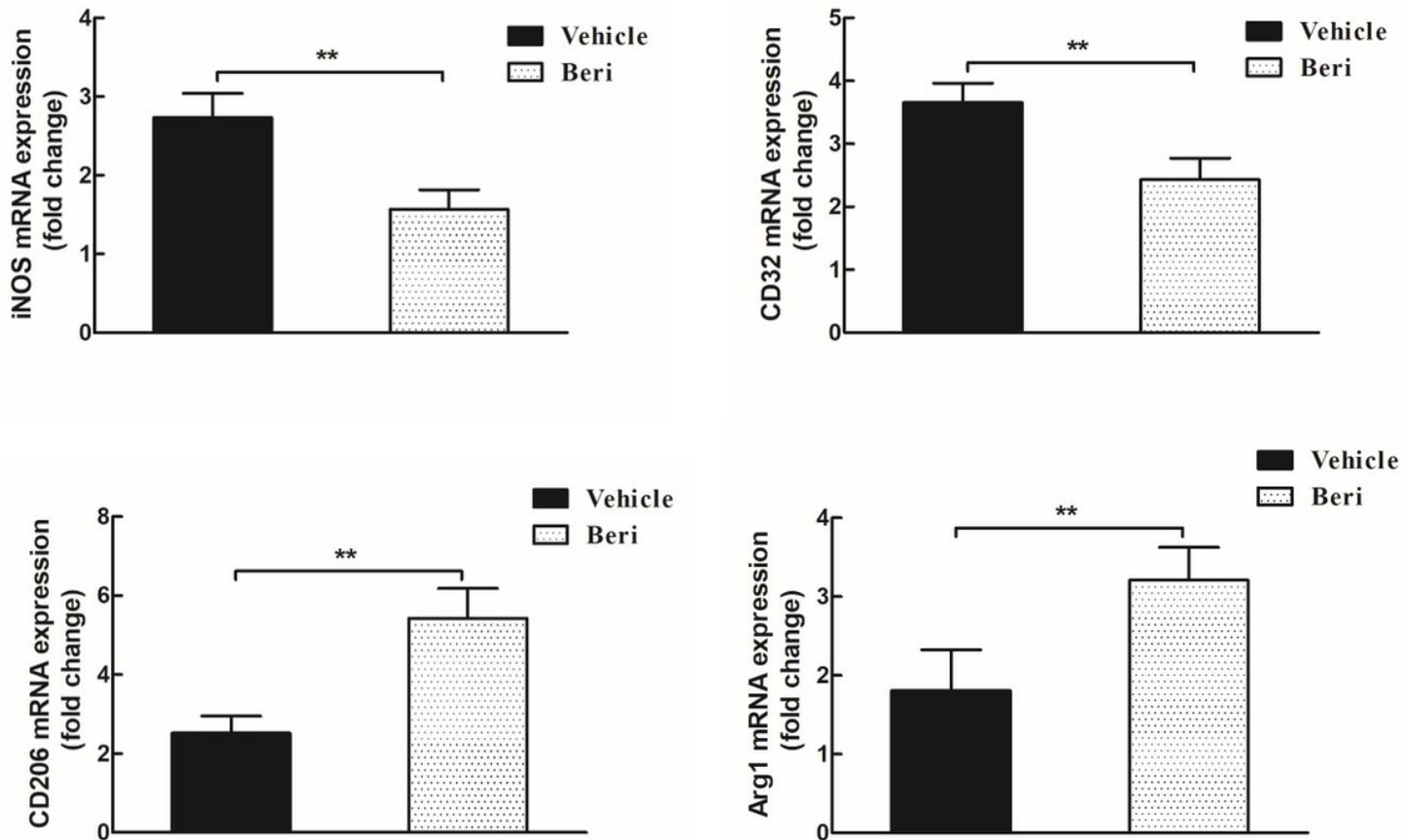


Figure 4

Treatment with Beri attenuated M1 markers expression and increased M2 markers expression in the perihematomal area after ICH. qPCR was used to examine the M1 markers expression (iNOS and CD32) and M2 markers (CD206 and Arg1). Values are presented as the mean \pm SD; * $p < 0.05$, ** $p < 0.01$ in all graphs.

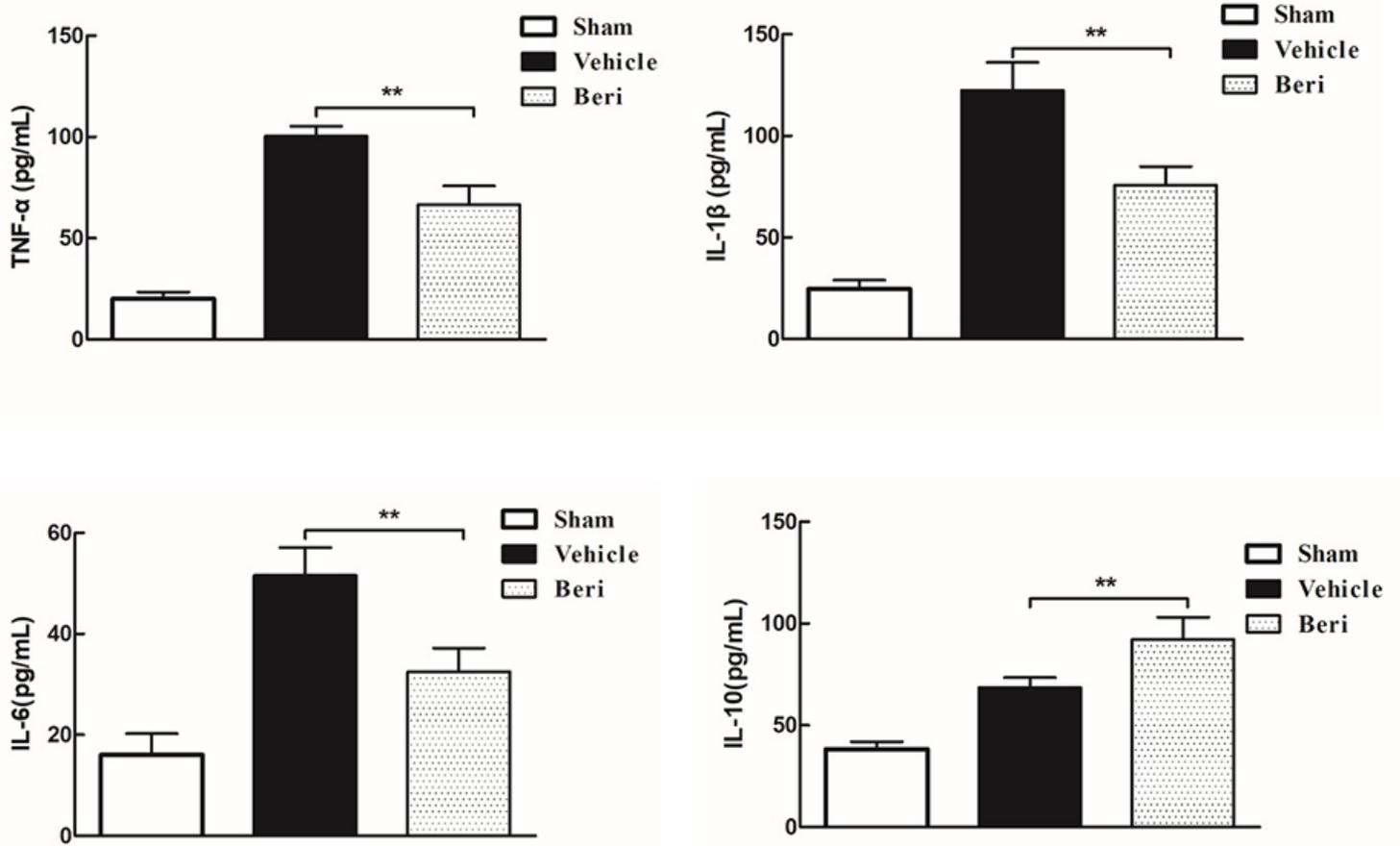


Figure 5

Treatment with Beri suppressed M1 proinflammatory cytokines releasing and increased M2 anti-inflammatory cytokines releasing in the perihematomal area after ICH. Elisa was used to examined the M1 proinflammatory cytokines (TNF- α , IL-1 β and IL-6) and M2 anti-inflammatory cytokines (IL-10). Values are presented as the mean \pm SD; * p <0.05, ** p <0.01 in all graphs.