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Original Article

Bindarit alleviates inflammation and early brain injury following experimental subarachnoid hemorrhage in rats

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Abstract: Bindarit is a small indazolic derivative which has shown anti-inflammatory and neuroprotective effects on multiple central nervous system (CNS) injuries. The effects of bindarit on subarachnoid hemorrhage (SAH) are not explored. This study was aimed to research the effects of bindarit on neuroinflammation and early brain injury (EBI) after experimental SAH in rats. Rats were randomly divided into sham, SAH, SAH+vehicle, and SAH+bindarit groups. Twenty-four hours after SAH, the SAH grade, neurological function, brain edema, blood brain barrier (BBB) permeability, cellular apoptosis, levels of inflammatory cytokines and microglial activation were evaluated. Results showed that administration of bindarit significantly ameliorated EBI including brain edema, BBB damage, cellular apoptosis and neurological deficits. In addition, bindarit also reduced inflammatory cytokine levels and microglial activation 24 h after SAH. In conclusion, the administration of bindarit might attenuate EBI following SAH through the downregulation of SAH-induced neuroinflammation.

Keywords: Bindarit, subarachnoid hemorrhage, early brain injury, inflammation

Introduction

Subarachnoid hemorrhage (SAH) is one of the most life-threatening diseases worldwide with high morbidity and mortality [1]. Despite the advances in medical technology, the prognosis of SAH is still poor. Cerebral vasospasm (CVS) which often occurs on the third day after SAH, reaches the peak on sixth and eighth day, and lasts for two or three weeks, has long been thought as the major cause of bad outcome after SAH [2, 3]. During the last several decades, studies of SAH have been mainly centered on CVS and it's sequelae. Although some encouraging results have been acquired from animal studies [4, 5], anti-vasospasm therapies are unable to improve the outcome of SAH in most clinical trials [6].

Recently, early brain injury (EBI) after SAH has been brought to the focus [7, 8]. EBI refers to the early injury to the brain which occurs in the first three days after SAH. Accumulating evidences suggest that EBI may play critical roles in the aggravation of neurological deficits and unfavorable prognosis after SAH [9]. The underlying pathophysiological mechanisms of SAH-induced EBI are not definitely clarified to date. Researches of the mechanisms of EBI after SAH have demonstrated that the early neuroinflammation is tightly associated with poor neurological grade on admission, malaise, fever, brain edema, increased blood brain barrier (BBB) permeability, thrombosis of small vessel, pathogenesis of vasospasm and delayed ischemic neurologic deficits [10-13].

Chemokines are a group of cytokines originally identified as mediator of leukocyte migration in inflammatory and immune responses [14]. In addition to their physiological action in healthy brain [15], chemokines increase in response to a various of pathological stimulations including multiple sclerosis, brain ischemia, Alzheimer's disease and SAH [16-18]. Among these chemokines, monocyte chemotactic protein-1 (MCP-1), also known as CCL-2, which is a CC chemokine specific for monocytes and produced

mainly by monocytes, vascular endothelial cells, smooth muscle cells and fibroblasts, has been found to be increased in the cerebrospinal fluid of patients with poorer prognosis after SAH [19]. Although MCP-1 is traditionally thought to act through the recruiting of monocyte infiltration, MCP-1 also results in neurological deficits through a mechanism in addition to, but separate from, it's classic role as a chemoattractant, because MCP-1 knockout model of ischemic injury has shown neuroprotective effects even before leukocyte infiltration [20]. Furthermore MCP-1 has been linked to the early microglial activation and inflammatory cytokine synthesis in a lipopolysaccharide (LPS)-induced neuroinflammation model of mice [21].

Bindarit is a small indazolic derivative that predominantly suppresses the transcription of the monocyte chemoattractant subfamily of CC chemokines [22]. Bindarit can inhibit the synthesis of MCP-1 and has displayed anti-inflammatory activities in a series of experimental models including arthritis, nephritis, colitis, pancreatitis and autoimmune encephalomyelitis [23-28].

The effects of bindarit on SAH-induced EBI have not been investigated. In this study, we hypothesized that bindarit might protect brain from EBI and improve neurological outcomes by attenuating neuroinflammation following SAH.

Material and methods

Animals

Experimental animal was Sprague-Dawley rats (280-350 g), afforded by animal experimental center of Zhejiang Chinese Medicine University. The use and care of animals employed in our model were approved by the Animal Care and Use Committee of Zhejiang Chinese Medicine University, in accordance with all relevant laws of China.

Rat SAH model

The SAH model was constructed by endovascular perforation as previously described [29]. Briefly, rats were anesthetized with a mixture of 3% isoflurane in 70%/30% medicalair/oxygen. A 4-0 nylon suture with sharpened tip was inserted into the right internal carotid artery and advanced until a resistance was felt (approximately 18-20 mm from the common carotid bifurcation). Then the suture was advanced for another 3 mm to perforate the right internal carotid artery close to the bifurcation with the middle cerebral artery to produce SAH. Sham group rats were also performed the similar procedures except the artery perforation.

After operation, the rats were returned to their cages with a room temperature of 23±1°C. Physical cooling (ice bag) was used to keep the rectal temperature at 37±0.5°C when required.

Preparation and administration of bindarit

Bindarit suspension of a concentration of 20 mg/mL was prepared in 0.5% methylcellulose (MTC) as previously described [30].

Rats were intraperitoneally given an injection of bindarit suspension (200 mg/kg) or MTC vehicle right after SAH. The injection was repeated with the same dose 12 h after SAH. Bindarit can reach the CNS parenchyma during the early stage of neuroinflammatory disease when the damage of BBB does not yet occur [28].

Assessment of SAH grade and neurological scores

Twenty-four hours after SAH, rats were sacrificed and brains were removed for the measurement of SAH grade. SAH grade was evaluated according to a grading system described previously [5] by an experimenter blinded to the study. Briefly, the system was based on the amount of subarachnoid blood clots distributed in the six segments of basal cistern: grade 1 (scores=0), no observable subarachnoid blood; grade 2 (scores=1), minimal subarachnoid clots; grade 3 (scores=2), moderate subarachnoid clots with recognizable arteries; and grade 4 (scores=3), blood clots covering all arteries. The total scores ranged from 0 to 18 was obtained by adding scores from all 6 segments.

Twenty-four hours after SAH, neurobehavioral scores were recorded according to the Garcia's method [29, 31] by an experimenter blinded to the study. Briefly, a 22 points scoring system was used to assess animal's neurological deficits by six aspects including spontaneous activity (0-3), reaction to side stroking (1-3), vibrissae touch (1-3), forepaw outstretching (0-3), climbing (0-3), limb symmetry (0-3), and beam walking ability (0-4). The beam walking ability

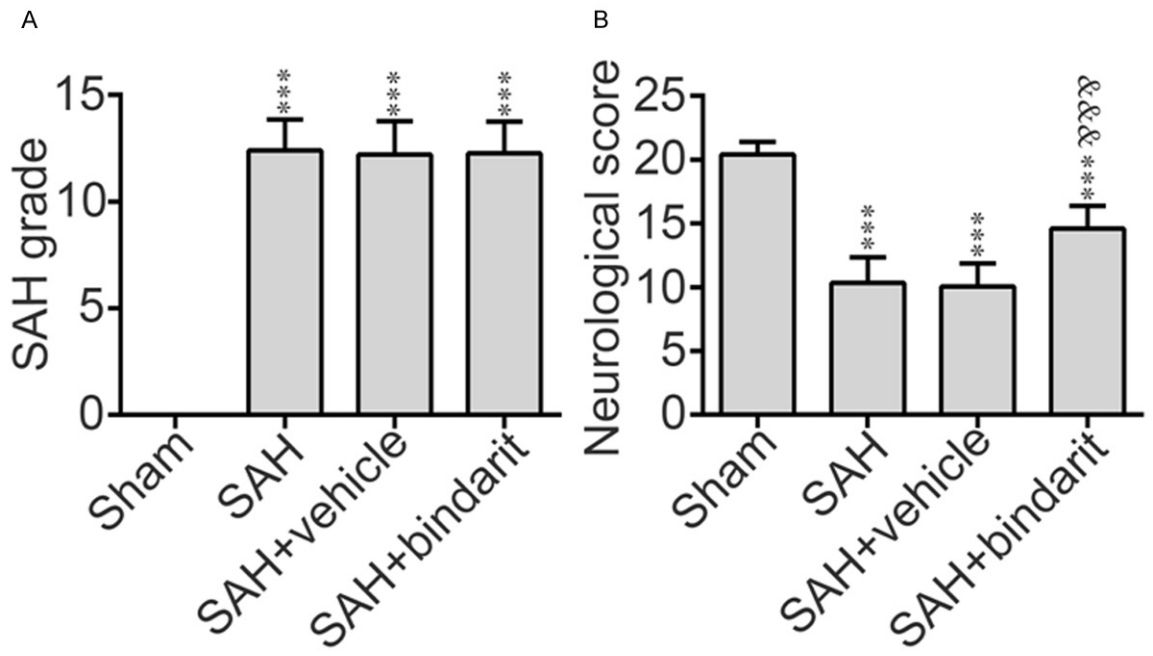

Figure 1. Effects of bindarit on SAH grade and neurological scores after SAH. Bindarit did not affect the bleeding level (A) bindarit attenuated neurological deficits 24 h after SAH (B) data were expressed as mean ± SD (***P<0.001 versus sham group, &&&P<0.001 versus SAH or SAH treated with vehicle).

refers to the walking distance on a wooden beam for 1 m. The minimum neurological score was 2 and the maximum was 22; a lower score represented more severe neurological deficits.

Brain water content

Brain edema was evaluated using the method described previously [32]. Briefly, the brain water content was calculated by formula (wet weight-dry weight/wet weight) ×100%. Rats were sacrificed 24 h after SAH and entire brain was quickly separated, placed into pre-weighed glass vial and weighed. Then brains were placed in an oven for 72 h at 100°C to obtain dry weight.

BBB permeability

BBB permeability was determined by Evans blue (EB) extravasation. Twenty-four hours after SAH, Evans blue dye (2 ml/kg) was intravenously injected, over a period of 2 min, allowing the dye to circulate for a total of 60 min [33]. Animals were re-anesthetized and subjected to transcardial perfusion with phosphate buffered saline (PBS), then brains were separated and homogenized in phosphate buffered saline. Trichloroacetic acid was then added to precipitate protein. After overnight incubation at 4°C, samples were centrifuged and the supernatant was used for spectrophotometric quantification of extravasated EB at 615 nm using a spectrophotometer.

TUNEL staining

Twenty-four hours after SAH, rats were perfused transcardially with 4% paraformaldehyde. Brains were removed, fixed in 4% paraformaldehyde and dehydrated with gradient sucrose solution. Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) staining was performed using an In Situ Cell Death Detection Kit (Roche Diagnostics, Mannheim, Germany) to detect in situ DNA fragmentation. Eight coronal sections of 8 μm were cut consecutively from bregma with 200 μm interval for TUNEL staining. Images were viewed with EVOS-fl digital inverted fluorescent microscopy. DAPI staining was performed according to routine laboratory methods. Five vision fields were randomly chosen for observation in each section. The mean number of TUNEL/DAPI double positive nuclei in the five views was thought as apoptotic index of each section. The apoptotic index of each brain was determined by the average percentage of double positive cells of the 8 sections.

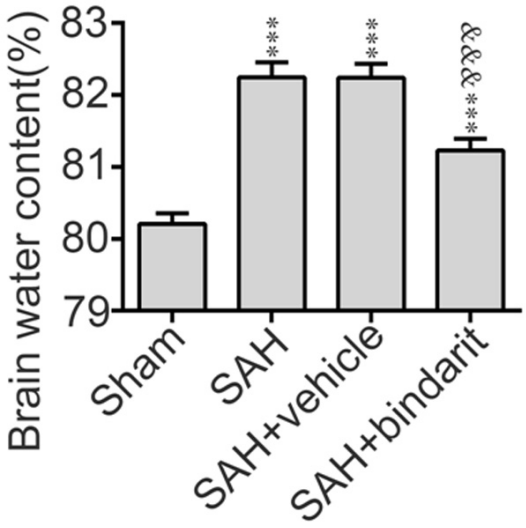

Figure 2. Effects of bindarit on brain edema after SAH. SAH induced increased brain water content (**Figure 2**). Bindarit attenuated brain water content 24 h after SAH (**Figure 2**). Data were expressed as mean ± SD (***P<0.001 versus sham group, &&&P<0.001 versus SAH group or SAH treated with vehicle).

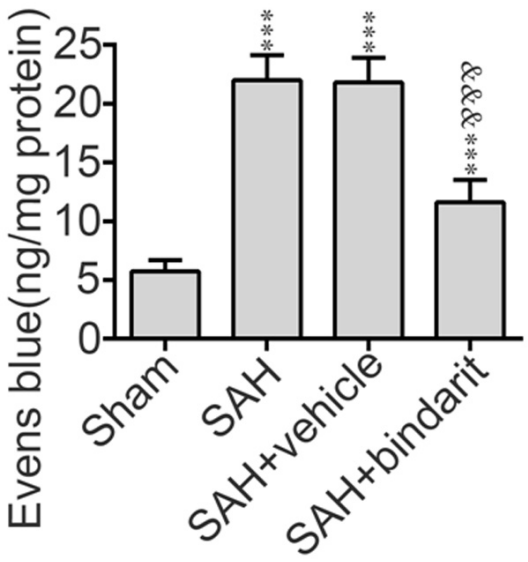

Figure 3. Effects of bindarit on BBB permeability after SAH. SAH induced increased extravasation of Evans blue (**Figure 3**). Bindarit decreased the Evans blue extravasation 24 h after SAH (**Figure 3**). Data were expressed as mean ± SD (***P<0.001 versus sham group, &&&P<0.001 versus SAH group or SAH treated with vehicle).

The numbers of apoptotic cells were counted with Image Pro Plus 6.0 Software (Media-Cybernetics, Bethesda, USA).

Western blot

Twenty-four hours after SAH, cortex of right hemisphere was obtained for western blot analysis. Samples were added with proteases inhibitor, supplemented with 2% beta-mercaptoethanol and 50 mM DTT and boiled for about 6 min. Proteins were separated on tris-glycine 4-15% acrylamide gels and transferred to PVDF membranes soaked in 5% nonfat milk in PBS-Tween 20 (0.05%) for 2 h. The proteins of caspase-3 and β-actin were examined by rabbit polyclonal antibody against caspase-3 (Santa Cruz, 1:500) and rabbit polyclonal antibody against β-actin (Santa Cruz, 1:500), then by incubation with secondary HRP-coupled antibody at room temperature. The densities of the bands were determined by the MiVnt image analysis system (Bio-Rad, Carlsbad, CA, USA).

Enzyme linked immunosorbent assay

ELISA kits specific for rats were used to determine the levels of inflammatory factors in brains according to the instructions (TNF-α from Diaclone Research, Besancon, France; IL-1β, IL-6 from BioSource Europe SA, Nivelles, Belgium).

Immunohistochemical examination

Immunohistochemistry for microglial CD11b was performed on 15 μm thick frozen coronal sections. Sections were incubated with rabbit monoclonal antibody against CD11b (Santa Cruz, 1:500) for 24 h at 4°C followed by incubation with secondary HRP-coupled antibody for 1 h at room temperature. Finally, sections were counterstained with haematoxylin and observed under a light microscope. Sections incubated in the absence of primary antibody were used as negative controls.

Statistical analysis

Results were expressed as the mean ± SD. One-way analysis of variance (ANOVA) was used for comparisons between multiple groups, followed by Turkey's test. P<0.05 was considered statistically significant.

Results

Effects of bindarit on SAH grade and neurological scores after SAH

Twenty-four hours after SAH, the grade score of SAH treated with bindarit (12.3±1.49) did not significantly differ from those of SAH group

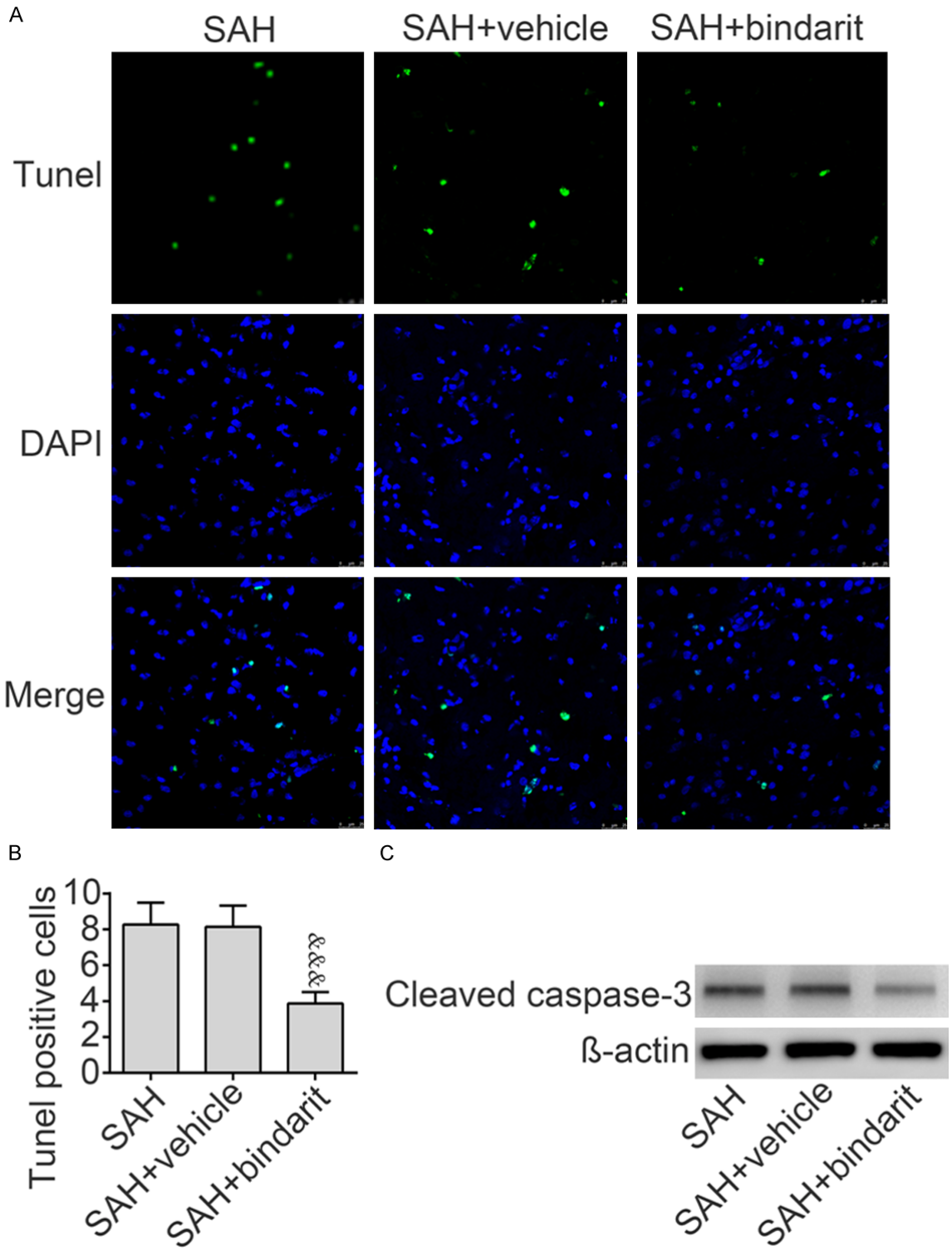

Figure 4. Effects of bindarit on cellular apoptosis after SAH. TUNEL staining showed bindarit administration reduced the cellular apoptosis 24 h after SAH (A, B) Western blot analysis showed bindarit decreased the expression of cleaved caspase-3 24 h after SAH (C) Nuclear morphology was indicated by DAPI staining and DNA breaks were detected by TUNEL analyses. Bar: 25 μm. Data were expressed as mean ± SD (&&&P<0.001 versus SAH or SAH treated with vehicle).

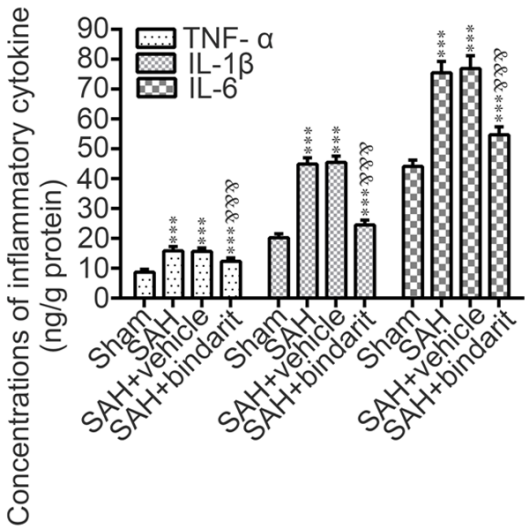

Figure 5. Effects of bindarit on the levels of inflammatory cytokines after SAH. Enzyme linked immunosorbent assay showed the levels of inflammatory cytokines, including TNF-α, IL-1β and IL-6, were significant increased in the SAH group (**Figure 5**). Bindarit attenuated the levels of inflammatory cytokines (**Figure 5**). Data were expressed as mean ± SD (***P<0.001 versus sham group, &&&P<0.001 versus SAH or SAH treated with vehicle).

(12.4±1.45) or SAH treated with vehicle (12.2±1.57) suggesting bindarit did not affect the bleeding level (**Figure 1A**). The neurological score was significantly decreased in the SAH group (10.3±2.02) compared with the sham group (20.4±0.99) (**Figure 1B**). After bindarit administration, neurological score was increased (14.6±1.76) compared with SAH group or SAH treated with vehicle (10.1±1.79) suggesting bindarit attenuated neurological deficits after SAH (**Figure 1B**).

Effects of bindarit on brain edema after SAH

Twenty-four hours after SAH, brain water content of SAH group (82.25±0.21%) was significantly higher than that of sham group (80.21±0.15%) indicating an early brain edema induced by SAH. Bindarit administration apparently attenuated brain water content (81.23±0.17%) compared with SAH group or SAH treated with vehicle (82.24±0.19%) (**Figure 2**).

Effects of bindarit on BBB permeability after SAH

Twenty-four hours after SAH, brains of SAH group showed a significantly increased BBB permeability to Evans blue (22.0±2.14 ng/mg protein) compared with the sham group (6.19±0.13 ng/mg protein) indicating an early corruption of BBB. Bindarit improved BBB permeability (11.6±1.92 ng/mg protein) as demonstrated by the decrease of Evans blue extravasation compared with the SAH group or SAH treated with vehicle (21.8±2.11 ng/mg protein) (**Figure 3**).

Effects of bindarit on cellular apoptosis after SAH

Twenty-four hours post injury, about 8.27± 1.22% cells in SAH group showed apoptosis (**Figure 4A**, **4B**). Bindarit significantly mitigated cellular apoptosis (3.87±0.64%) compared with SAH group or SAH treated with vehicle (8.13±1.19%) (**Figure 4A**, **4B**). Western blot analysis showed the expression of cleaved caspase-3 in SHA group significantly increased compared with the sham group. Bindarit administration downregulated caspase-3 expression after SAH compared with the SAH group or SAH treated with vehicle (**Figure 4C**).

Effects of bindarit on the levels of inflammatory cytokines after SAH

Enzyme linked immunosorbent assay showed the levels of inflammatory cytokines including TNF-α, IL-1β and IL-6, were significantly increased in the SAH group (15.9±1.46, 44.9± 2.17 and 75.5±3.74 ng/mg protein to TNF-α, IL-1β and IL-6 respectively) 24 h after SAH. Bindarit administration dramatically attenuated the levels of inflammatory cytokines (12.4± 1.06, 24.5±1.69 and 54.8±2.60 ng/mg protein to TNF-α, IL-1β and IL-6 respectively) compared with SAH group or SAH treated with vehicle (15.6±1.19, 45.5±2.07 and 76.9±4.29 ng/mg protein to TNF-α, IL-1β and IL-6 respectively) suggesting the anti-inflammatory effects of bindarit (**Figure 5**).

Effects of bindarit on the activation of microglia after SAH

Immunochemistry showed the microglias increased in number and became activated altering their morphology with upregulation of CD11b 24 h after SAH. Bindarit administration dramatically inhibited CD11b positive microglia activation (**Figure 6**).

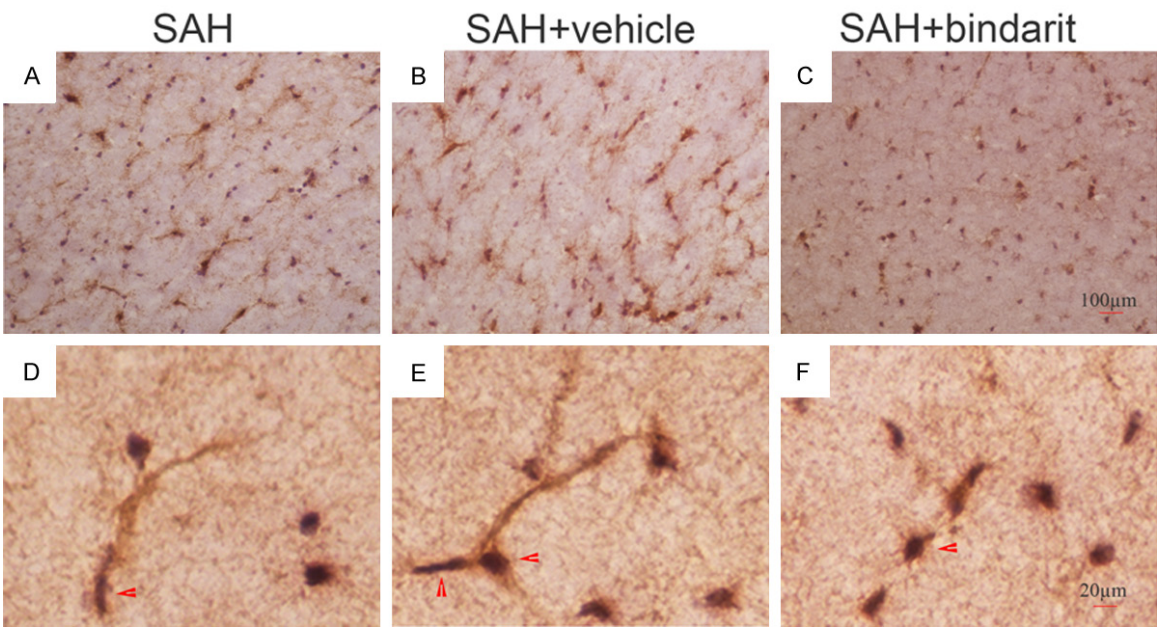

Figure 6. Effects of bindarit on the activation of microglia after SAH. Immunochemistry showed the microglias increased in number and became activated, altering their morphology with upregulating of CD11b 24 h after SAH. CD11b staining for microglias showed bindarit inhibited microglia activation in the brain after SAH (**Figure 6**). Red arrow: microglia. A-C: Bar: 100 μm. D-F: Bar: 20 μm.

Discussion

Increasing evidences suggest that inflammation plays important role in SAH-induced early and delayed brain injury, and contributes to the poor outcome of SAH [10-13]. Monocyte chemoattractant proteins (MCPs), which belong to the beta chemokine family, play pathological roles in SAH due to their pro-inflammatory effects. Among them, MCP-1 (CCL2) and it's receptor CCR2 have been proved to play an important role in SAH and other neuroinflammatory injuries, contributing to the neuronal death and cerebral dysfunction [19]. Thus it stand to reason to use anti-inflammatory drugs targeted chemokine synthesis to prevent either early or delaying aggravation of SAH-induced brain damage. Especially, drugs able to inhibit MCP-1 synthesis might have great potential value of suppressing the chemokine-induced neuroinflammation in the pathophysiology of SAH.

Considering the success of the MCP-1 synthesis inhibitor bindarit on alleviating several human clinical conditions and animal models associated with neuroinflammation, initial study was performed to examine it's potential effects on neuroinflammation and EBI after SAH. The main findings of the present study are: 1) after bindarit administration, EBI such as neurological score, brain edema, BBB permeability damage and neuronal apoptosis are improved after SAH; 2) after bindarit administration, the levels of inflammatory cytokines and microglial activation are suppressed after SAH. These findings suggest for the first time that bindarit may alleviate the EBI through the inhibition of SAH-induced inflammation.

How bindarit inhibited SAH-induced neuroinflammation was not clarified in this study. Our study showed that the levels of inflammatory cytokines such as TNF-α, IL-1β and IL-6 were greatly reduced after bindarit treatment. The underlying mechanisms of the bindarit-induced reduction of inflammatory cytokines were not fully explored in this study. The inhibition of microglial activation after bindarit administration might contribute to this effect as microglial cells are the resident macrophages of the central nervous system (CNS). After SAH, especially on acute stage, activated microglias provoke excessive secretion of pro-inflammatory cytokines and contribute to the development of brain edema, BBB disruption and secondary neuronal injury after SAH [1]. The pro-inflammatory cytokines secreted by activated microglial

cells include IL-6, IL-1β and TNF-α [34] and all of them have been found to be increased early after SAH and strongly linked to the brain injury in both patients and animals [35-37]. The activated microglia is also linked to the migration of microglias from other regions of the brain, proliferation of resident microglias and infiltration of blood monocytes [38]. This microglia-induced recruitment of monocytes from peripheral blood into the CNS has been linked to the MCP-1 expressed on microglia [39, 40]. Microglial MCP-1 also controls the proliferation, recruitment and activation of microglias [41-43], thus potentially supports a self-sustaining cycle of neuroinflammation. It has been revealed that bindarit significantly suppresses MCP-1 expression on microvascular endothelial cells, microglias and astrocytes in brain microvascular and parenchymal compartments. While all these cell types respond with significant reduction in MCP-1 mRNA after bindarit administration, microglia is the most sensitive one [28]. In addition, MCP-1 harbors a α-helixin it's C-terminal which is a binding site for GAGs typically found on the luminal endothelial surface [44]. MCP-1 binds to the luminal surface of cultured endothelial cells and induces a firm adhesion and transmigration of mononuclear leukocytes [45, 46].

Taken together, in the present study, bindarit administration appears to have a multiple anti-inflammation properties in EBI after SAH. Certainly, other cell types and mechanisms might also be involved in the bindarit-induced anti-inflammatory and neuroprotective effects in EBI after SAH. These need to be further explored in our future study.

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Disclosure of conflict of interest

None.

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References

[1] Sehba FA, Hou J, Pluta RM and Zhang JH. The importance of early brain injury after subarachnoid hemorrhage. Prog Neurobiol 2012; 97: 14-37.

[2] Wilkins RH. Cerebral vasospasm. Crit Rev Neurobiol 1990; 6: 51-77.

[3] Dorsch NW. Cerebral arterial spasm--a clinical review. Br J Neurosurg 1995; 9: 403-412.

[4] Hong Y, Guo S, Chen S, Sun C, Zhang J and Sun X. Beneficial effect of hydrogen-rich saline on cerebral vasospasm after experimental subarachnoid hemorrhage in rats. J Neurosci Res 2012; 90: 1670-1680.

[5] Sugawara T, Ayer R, Jadhav V, Chen W, Tsubokawa T and Zhang JH. Simvastatin attenuation of cerebral vasospasm after subarachnoid hemorrhage in rats via increased phosphorylation of Akt and endothelial nitric oxide synthase. J Neurosci Res 2008; 86: 3635-3643.

[6] Wang X, Li YM, Li WQ, Huang CG, Lu YC and Hou LJ. Effect of clazosentan in patients with aneurysmal subarachnoid hemorrhage: a meta-analysis of randomized controlled trials. PLoS One 2012; 7: e47778.

[7] Broderick JP, Brott TG, Duldner JE, Tomsick T and Leach A. Initial and recurrent bleeding are the major causes of death following subarachnoid hemorrhage. Stroke 1994; 25: 1342-1347.

[8] Frykholm P, Andersson JL, Långström B, Persson L and Enblad P. Haemodynamic and metabolic disturbances in the acute stage of subarachnoid haemorrhage demonstrated by PET. Acta Neurol Scand 2004; 109: 25-32.

[9] Kusaka G, Ishikawa M, Nanda A, Granger DN and Zhang JH. Signaling pathways for early brain injury after subarachnoid hemorrhage. J Cereb Blood Flow Metab 2004; 24: 916-925.

[10] Chaichana KL, Pradilla G, Huang J and Tamargo RJ. Role of inflammation (leukocyte-endothelial cell interactions) in vasospasm after subarachnoid hemorrhage. World Neurosurg 2010; 73: 22-41.

[11] Kaynar MY, Tanriverdi T, Kafadar AM, Kacira T, Uzun H, Aydin S, Gumustas K, Dirican A and Kuday C. Detection of soluble intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in both cerebrospinal fluid and serum of patients after aneurysmal subarachnoid hemorrhage. J Neurosurg 2004; 101: 1030-1036.

[12] Kubo Y, Ogasawara K, Kakino S, Kashimura H, Tomitsuka N, Sugawara A and Ogawa A. Serum inflammatory adhesion molecules and high-sensitivity C-reactive protein correlates with delayed ischemic neurologic deficits after sub-

arachnoid hemorrhage. Surg Neurol 2008; 69: 592-596.
[13] Mack WJ, Mocco J, Hoh DJ, Huang J, Choudhri TF, Kreiter KT, Lozier A, Opperman M, Poisik A, Yorgason J, Solomon RA, Mayer SA and Connolly ES. Outcome prediction with serum intercellular adhesion molecule-1 levels after aneurysmal subarachnoid hemorrhage. J Neurosurg 2002; 96: 71-75.
[14] Luster AD. Chemokines--chemotactic cytokines that mediate inflammation. N Engl J Med 1998; 338: 436-445.
[15] Hesselgesser J and Horuk R. Chemokine and chemokine receptor expression in the central nervous system. J Neurovirol 1999; 5: 13-26.
[16] Asensio VC and Campbell IL. Chemokines in the CNS: plurifunctional mediators in diverse states. Trends Neurosci 1999; 22: 504-512.
[17] Rubio-Perez JM and Morillas-Ruiz JM. A review: inflammatory processin Alzheimer's disease, role of cytokines. ScientificWorldJournal 2012; 2012: 756357.
[18] Lad SP, Hegen H, Gupta G, Deisenhammer F and Steinberg GK. Proteomic biomarker discovery in cerebrospinal fluid for cerebral vasospasm following subarachnoid hemorrhage. J Stroke Cerebrovasc Dis 2012; 21: 30-41.
[19] Kim GH, Kellner CP, Hahn DK, Desantis BM, Musabbir M, Starke RM, Rynkowski M, Komotar RJ, Otten ML, Sciacca R, Schmidt JM, Mayer SA and Connolly ES Jr. Monocyte chemoattractant protein-1 predicts outcome and vasospasm following aneurysmal subarachnoid hemorrhage. J Neurosurg 2008; 109: 38-43.
[20] Hughes PM, Allegrini PR, Rudin M, Perry VH, Mir AK and Wiessner C. Monocyte chemoattractant protein-1 deficiency is protective in a murine stroke model. J Cereb Blood Flow Metab 2002; 22: 308-317.
[21] Rankine EL, Hughes PM, Botham MS, Perry VH and Felton LM. Brain cytokine synthesis induced by an intraparenchymal injection of LPS is reduced in MCP-1-deficient mice prior to leucocyte recruitment. Eur J Neurosci 2006; 24: 77-86.
[22] Mirolo M, Fabbri M, Sironi M, Vecchi A, Guglielmotti A, Mangano G, Biondi G, Locati M and Mantovani A. Impact of the anti-inflammatory agent bindarit on the chemokinome: selective inhibition of the monocyte chemotactic proteins. Eur Cytokine Netw 2008; 19: 119-122.
[23] Zoja C, Corna D, Benedetti G, Morigi M, Donadelli R, Guglielmotti A, Pinza M, Bertani T and Remuzzi G. Bindarit retards renal disease and prolongs survival in murine lupus autoimmune disease. Kidney Int 1998; 53: 726-734.
[24] Guglielmotti A, D'Onofrio E, Coletta I, Aquilini L, Milanese C and Pinza M. Amelioration of rat adjuvant arthritis by therapeutic treatment with bindarit, an inhibitor of MCP-1 and TNF-alpha production. Inflamm Res 2002; 51: 252-258.
[25] Bhatia M, Ramnath RD, Chevali L and Guglielmotti A. Treatment with bindarit, a blocker of MCP-1 synthesis, protects mice against acute pancreatitis. Am J Physiol Gastrointest Liver Physiol 2005; 288: G1259-1265.
[26] Bhatia M, Landolfi C, Basta F, Bovi G, Ramnath RD, de Joannon AC and Guglielmotti A. Treatment with bindarit, an inhibitor of MCP-1 synthesis, protects mice against trinitrobenzene sulfonic acid-induced colitis. Inflamm Res 2008; 57: 464-471.
[27] Rulli NE, Rolph MS, Srikiatkhachorn A, Anantapreecha S, Guglielmotti A and Mahalingam S. Protection from arthritis and myositis in a mouse model of acute chikungunya virus disease by bindarit, an inhibitor of monocyte chemotactic protein-1 synthesis. J Infect Dis 2011; 204: 1026-1030.
[28] Ge S, Shrestha B, Paul D, Keating C, Cone R, Guglielmotti A and Pachter JS. The CCL2 synthesis inhibitor bindarit targets cells of the neurovascular unit, and suppresses experimental autoimmune encephalomyelitis. J Neuroinflammation 2012; 9: 171.
[29] Hasegawa Y, Suzuki H, Altay O and Zhang JH. Preservation of tropomyosin-related kinase B (TrkB) signaling by sodium orthovanadate attenuates early brain injury after subarachnoid hemorrhage in rats. Stroke 2011; 42: 477-483.
[30] Grassia G, Maddaluno M, Guglielmotti A, Mangano G, Biondi G, Maffia P and Ialenti A. The anti-inflammatory agent bindarit inhibits neointima formation in both rats and hyperlipidaemic mice. Cardiovasc Res 2009; 84: 485-493.
[31] Garcia JH, Wagner S, Liu KF and Hu XJ. Neurological deficit and extent of neuronal necrosis attributable to middle cerebral artery occlusion in rats. Statistical validation. Stroke 1995; 26: 627-634.
[32] Roof RL, Duvdevani R, Heyburn JW and Stein DG. Progesterone rapidly decreases brain edema: treatment delayed up to 24 hours is still effective. Exp Neurol 1996; 138: 246-251.
[33] Fujii M, Duris K, Altay O, Soejima Y, Sherchan P and Zhang JH. Inhibition of Rho kinase by hydroxyfasudil attenuates brain edema after subarachnoid hemorrhage in rats. Neurochem Int 2012; 60: 327-333.
[34] Sivakumar V, Foulds WS, Luu CD, Ling EA and Kaur C. Retinal ganglion cell death is induced by microglia derived pro-inflammatory cytokines in the hypoxic neonatal retina. J Pathol 2011; 224: 245-260.
[35] Gaetani P, Tartara F, Pignatti P, Tancioni F, Rodriguez y Baena R and De Benedetti F. cis-

ternal CSF levels of cytokines after subarachnoid hemorrhage. Neurol Res 1998; 20: 337-342.

[36] Greenhalgh AD, Brough D, Robinson EM, Girard S, Rothwell NJ and Allan SM. Interleukin-1 receptor antagonist is beneficial after subarachnoid haemorrhage in rat by blocking haem-driven inflammatory pathology. Dis Model Mech 2012; 5: 823-833.

[37] Larysz-Brysz M, Lewin-Kowalik J, Czuba Z, Kotulska K, Olakowska E, Marcol W, Liśkiewicz A and Jędrzejowska-Szypułka H. Interleukin-1β increases release of endothelin-1 and tumor necrosis factor as well as reactive oxygen species by peripheral leukocytes during experimental subarachnoid hemorrhage. Curr Neurovasc Res 2012; 9: 159-166.

[38] Bechmann I, Goldmann J, Kovac AD, Kwidzinski E, Simbürger E, Naftolin F, Dirnagl U, Nitsch R and Priller J. Circulating monocytic cells infiltrate layers of anterograde axonal degeneration where they transform into microglia. FASEB J 2005; 19: 647-649.

[39] D'Mello C, Le T and Swain MG. Cerebral microglia recruit monocytes into the brain in response to tumor necrosis factoralpha signaling during peripheral organ inflammation. J Neurosci 2009; 29: 2089-2102.

[40] Yao H, Yang Y, Kim KJ, Bethel-Brown C, Gong N, Funa K, Gendelman HE, Su TP, Wang JQ and Buch S. Molecular mechanisms involving sigma receptor-mediated induction of MCP-1: implication for increased monocyte transmigration. Blood 2010; 115: 4951-4962.

[41] Deng YY, Lu J, Ling EA and Kaur C. Monocyte chemoattractant protein-1 (MCP-1) produced via NF-kappaB signaling pathway mediates migration of amoeboid microglia in the periventricular white matter in hypoxic neonatal rats. Glia 2009; 57: 604-621.

[42] Hinojosa AE, Garcia-Bueno B, Leza JC and Madrigal JL. CCL2/MCP-1 modulation of microglial activation and proliferation. J Neuroinflammation 2011; 8: 77.

[43] Thacker MA, Clark AK, Bishop T, Grist J, Yip PK, Moon LD, Thompson SW, Marchand F and McMahon SB. CCL2 is a key mediator of microglia activation in neuropathic pain states. Eur J Pain 2009; 13: 263-272.

[44] Chakravarty L, Rogers L, Quach T, Breckenridge S and Kolattukudy PE. Lysine 58 and histidine 66 at the C-terminal alpha-helix of monocyte chemoattractant protein-1 are essential for glycosaminoglycan binding. J Biol Chem 1998; 273: 29641-29647.

[45] Gerszten RE, Garcia-Zepeda EA, Lim YC, Yoshida M, Ding HA, Gimbrone MA Jr, Luster AD, Luscinskas FW and Rosenzweig A. MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. Nature 1999; 398: 718-723.

[46] Hardy LA, Booth TA, Lau EK, Handel TM, Ali S and Kirby JA. Examination of MCP-1 (CCL2) partitioning and presentation during transendothelial leukocyte migration. Lab Invest 2004; 84: 81-90.