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Original Article

Protective effects of histone deacetylase inhibition by Scriptaid on brain injury in neonatal rat models of cerebral ischemia and hypoxia

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Abstract: Background: Neonatal hypoxia-ischemia brain damage (HBID) can cause a series of neurological sequelae, such as movement and cognitive impairment, and there is currently no clinically effective treatment. Changes in epigenetic processes had been shown to be involved in the development of a series of neurodegenerative diseases, and HDAC inhibition by Scriptaid had been shown to reduce severe traumatic brain injury by suppressing inflammatory responses. This study investigated the protective effect of HDAC inhibition by Scriptaid after HBID. Methods: We established the neonatal rat HBID model, and used intraperitoneal injection of HDAC inhibitor scriptaid as a treatment. 7 days after HBID, nuclear magnetic resonance imaging (MRI) was used to detect infarct volume. The otarod test, wire hang test and Morris water maze were used to evaluate the HBID model of neurobehavioral dysfunction. Immunoblotting, immunofluorescence, and quantitative real-time PCR (RT-qPCR) were used to detect gene expression. Results: HDAC inhibition by Scriptaid treatment could not only reduce the infarct volume and neuronal degeneration in HBID rats, but also helped to improve their neurobehavioral dysfunction. 7 days after HBID, the expression of HDAC-1, HDAC-2 and HDAC-3 in the infarct volume of HBID + Veh group rats were much more than that in sham group ($P<0.05$), but Scriptaid could significantly inhibit those expression ($P<0.05$), and significantly increased the acetylation of H3 and H4 in HBID rats. In vivo and vitro results demonstrated that Scriptaid had no significant effect on oligodendrocyte MBP protein expression after OGD, but Scriptaid -treated microglia cultures had protective effects on OGD-treated OLG, M1 microglia suppressed OLG activity after OGD, and M2 enhanced its activity. In vivo experiments at 7 days after HBIDI injury showed that Scriptaid could promote the polarization of microglia into M2 microglia, reduced the expression of pro-inflammatory factors, and enhance the expression of anti-inflammatory cytokines. Conclusion: After HBID, HDAC inhibitor Scriptaid inhibits inflammatory responses and protects the brain by promoting the polarization of microglia in brain tissue to M2 microglia.

Keywords: Histone deacetylase inhibition, hypoxia-ischemia brain damage, Inflammation, microglial polarization, oligodendrocyte

Introduction

Hypoxic-ischemic brain damage (HIBD) is the brain damage caused by suffocation during the perinatal period in newborns, and is one of the leading causes of neonatal death [1].

HBID can cause a series of neurological sequelae, such as movement and cognitive impairment, but there is currently no clinically effective treatment [2]. Research to study the pathogenesis of HIBD put forward many theories, such as free radical ion production, NO production, inflammatory response, excitotoxicity, and ion imbalance [3, 4], but it has not been clarified so far.

Various studies in recent years had shown that changes in epigenetic processes were involved in the development of a series of neurodegenerative diseases, such as Alzheimer's disease [5], Parkinson's disease [6], epilepsy [7], Friedrich's ataxia [8], Huntington's disease [9], etc. Neurodegenerative disease is a type of chronic progressive disease with loss of specific neuron function, and the occurrence of these diseases is related to the imbalance in histone transcriptional dysfunction and acetylation levels. The acetylation status of histones is determined by the combination of histone acetyltransferase (HAT) and histone deacetylase (HDAC). Histone acetyltransferases affect the

level of acetylation of histones, loosen the chromatin structure, and promote transcription. However, histone deacetylase catalyzes the deacetylation of histones, promotes chromatin condensation, and inhibits the transcription of target genes. Histone deacetylases play an extremely important role in regulating acetylation levels and affecting chromatin tightness [10]. Previous studies have shown that deacetylation of histones plays a role in cognitive and memory functions as well as neurological disorders and diseases [11]. After brain injury, inhibition of histone deacetylase activity could activate the transcription factor Nrf2 [12], regulate the polarization state of microneuronal cells/macrophages [13], and suppress inflammatory responses [14] to exert brain protection. The purpose of treating neurological-related diseases can be achieved by reversing the function of HDAC.

Therefore, our study focused on whether HDAC inhibitors have a protective effect on brain tissue in HIBD. Our study focused on the application of scriptaid, a potent and often used for research histone deacetylase (HDAC) inhibitor, in the treatment of HBID rat models. We explored the protective effects of HDAC inhibition on brain injury in neonatal rat models of cerebral ischemia and hypoxia. In this paper, we found that HDAC inhibitor Scriptaid inhibits inflammatory responses and protects the brain by promoting the polarization of microglia in brain tissue to M2 microglia after HBID. All in all, our research provides a new possibility for the treatment of HBID.

Materials and methods

Experimental animals

Adult male and female SD rats were mixed feeding in a standard animal room (24 ± 1°C, a humidity of 50 ± 10%, natural lighting conditions, ensuring adequate food and drinking water). After the female rats were pregnant and spontaneously delivered, the pups and their mother rats were individually fed in a large cage. We selected 7-day-old newborn SD rats (male and female, weight 11-18 g) as experimental animals. The present study was performed with the approval of the Ethics Committee of the Third People's Hospital of Qingdao.

Randomization of animal grouping

7-day-old newborn SD rats were randomly divided into Sham group, HBID + Veh group, and HBID + Scri group. The rats in HBID + Scri group were immediately intraperitoneally injected with 3.5 mg/kg scriptaid, and the same dose of 0.5% DMSO solution in saline for HBID + Veh group rats. This was repeated daily until the end of the experiment.

HBID model

According to Rice-Vannucci [15], we established a newborn rat HIBD model. Rats were anesthetized by intraperitoneal injection of 350 mg/kg of 4% chloral hydrate (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China). After anesthetized rats were disinfected with alcohol, a longitudinal incision of approximately 1 cm was made in the middle of the neck, the left common carotid artery was identified and separated, and bilateral ligatures from the 6th surgical line were used. After the blood vessel was cut, the wound was quickly sutured and applied. After the operation, the pups were sent back to the original mother rats. After natural breast-feeding for 4 h, they were placed in an organic glass anoxic box 35 cm long, 15 cm wide, and 20 cm high. A small amount of sodium lime was placed at the bottom of the hypoxia tank and the cap was tightened. After being placed in a 37°C constant temperature water bath, a mixture of 8% oxygen and 92% nitrogen was input, and the flow rate was controlled at 1-2 L/min for 2 hours. After the end of hypoxia, we put pups back to the original mother. In the sham group, only the left common carotid artery was isolated but the common carotid artery was not ligated, and no hypoxic operation was performed.

Drug administration

Scriptaid (s7817, Sigma-Aldrich, USA) was stored at 100 mg/ml in DMSO, and diluted 0.5 mg/ml with sterile saline, boiled and cooled to below 37°C and injected at a dose of 3.5 mg/kg intraperitoneally.

Cerebral infarction volume determination

Magnetic resonance imaging (MRI) was used to detect the infarct volume. Rats were injected peritoneally with 4% chloral hydrate by 350

mg/kg to be anesthetized. The Phillips GyroscanIntera 1.5TMRI (Phillips, Eindhoven, Netherlands) and the Rat coil (Shanghai Chenguang Medical Technology Co., Ltd., Shanghai, China) were used for FSE T2W imaging. Parameter: TR was 1600 ms, TE was 80 ms, slice thickness was 1 mm, slice gap was 0, FOV was 35 mm × 35 mm, matrix was 256 × 256. And Image J software (National Institutes of Health, Maryland, USA) was used to measure the area of cerebral infarction per layer. The percentage of cerebral infarction volume was calculated by the following formula [16]. The percentage of cerebral infarction volume = (S1 + S2 + S3 + SN)H/(S1 total + S2 total + S3 total + SN total), S1~SN was the infarct size of each layer, and S1 total~SN total was the area of brain tissue in each layer, and H was slice thickness.

Neurobehavioral tests

Rotarod test: Rotary rod test was performed as described previously [17]. In short, the rat was placed on a rotating drum and accelerated from 4 to 40 revolutions in 5 minutes. The time that the animal was off the drum was recorded. The test was started on the day before the HBID and the animals were tested five times. The average was these trials as each pre-operative baseline animal. After surgery, animals were tested five times a day for up to 7 days.

Wire hang test: Wire hang test was performed as described previously [18]. The experimental apparatus was a stainless steel bar (50 cm long, 2-mm diameter) resting on two vertical supports and elevated 37 cm above a flat surface. Rats were placed on the bar midway between the supports and were observed for 30 s in four trials. The amount of time spent hanging was recorded and scored according to the following criteria: 0, Rat shedding; 1, rats hung on the bar with two front paws; 2, rats hung on the bar and tried to climb the bar; 3, rats hung onto the bar with two fore paws and one or both hind paws; 4, rats hung on the bar with all four paws and its tail wrapped around the bar; 5, rats fled to one of the supports.

Morris water maze

Positioning navigation test: The rats faced the pool wall and entered the water at random from four different quadrants. The time that the rats entered the water to find the underwater platform was recorded, i.e. the escape latency. If the rat fails to find the platform within 120 s, the rat is led to the platform with a wooden stick and stays for 10 s. The escape latency of this quadrant is recorded as 120 s. The above quadrant training is repeated. After the test was completed, the water on the rat body is dried with a dry towel, and it is put back into the cage. All rats were continuously trained for 5 days, every morning and afternoon, and the time that was required for rat to find the platform in 4 quadrants was recorded, and the average of twice time in 4 quadrant latency was recorded as the final result of rat for the day.

Space exploration test: On the afternoon of the 5th day, the platform was removed. The rat was randomly entered into the water from the midpoint of a quadrant wall and the number of times that rat swam through the original platform position within 120 s were recorded, i.e. number of traversings of the platform.

Primary microglia/oligodendrocyte cultures

Primary Microglia/Oligodendrocyte separation and culture was as described as previously [19, 20]. In our study, M0 was means that microglia were incubated with 1 uM Scriptaid or equal amount of solvent for 48 hours; M1 was means that microglia were incubated with 100 ng/mL LPS (L2880, Sigma-Aldrich, USA) plus 20 ng/mL IFN-γ (I4777, Sigma-Aldrich, USA) for 48 hours; M2 means that microglia were incubated with 20 ng/mL IL-4 (SRP3211, Sigma-Aldrich, USA) for 48 hours [20].

Cell viability and death

The cell viability was measured by MTT assay. MTT (1 mg/mL) was added to living cells treated with different methods and incubated at 37°C for 2 hours. Then the cell culture medium was carefully removed and 100 μl/well of DMSO was added at 37°C for 15 min. While gently shaking the plate to better dissolve the crystals, absorbance was measured at OD490 nm using a microplate reader.

The LDH content of the culture fluid was used to characterize cell death. LDH Assay Kit/Lactate Dehydrogenase Assay Kit (Colorimetric) (ab102526, Abcam, UK) was used to detect the LDH content in cell cultures.

Table 1. RT-qPR primers

Gene	Primer sequence (5'-3')
CD16	F: AATGCACACTCTGGAAGCCAA
	R: CACTCTGCCTGTCTGCAAAAG
CD206	F: CTCTGTTCAGCTATTGGACGC
	R: TGGCACTCCCAAACATAATTTGA
TNF-α	F: CAGGCGGTGCCTATGTCTC
	R: CGATCACCCCGAAGTTCAGTAG
IFN-γ	F: GCCACGGCACAGTCATTGA
	R: TGCTGATGGCCTGATTGTCTT
IL-13	F: TGAGCAACATCACACAAGACC
	R: GGCCTTGCGGTTACAGAGG
IL-4	F: GGTCTCAACCCCCAGCTAGT
	R: GCCGATGATCTCTCTCAAGTGAT
GAPDH	F: GATGAACCTAAGCTGGGACCC
	R: TGTGAACGGATTTGGCCGTA

Real-time fluorescence quantitative PCR

Trizol was used to extract the total RNA of the tissue or cell. The extracted RNA was reverse transcribed into cDNA by using PrimeScript™RT Master Mix reverse transcription kit (RR036B, Takara, Beijing, China). PCR parameters set: 37°C/60 minutes, 85°C/5 seconds. 20 μl Real-time fluorescence quantitative PCR (RT-qPCR) system was prepared according to the SYBR Green qPCR Master Mix kit instructions (638320, TakaRa, Beijing, China) and amplified using ABI 7500 fluorescence quantitative PCR instrument (Applied Biosystems, Maryland, USA). PCR parameters set: 95°C/30 s, [90°C/5 s, 65°C/30 s] -40 cycles. Primer for RT-qPCR is shown in **Table 1**.

Western blot

Tissue or cell lysates were separated by SDS-page and then transferred to PVDF membrane. Primary antibody was selected as follows: anti-HDAC1 (ab19845, 1:1000), or anti-HDAC2 (ab32117, 1:1000), or anti-HDAC3 (ab19845, 1:1000), or anti-acetyl-H3 (ab52946, 1:500), anti-acetyl-H4 (ab109463, 1:1500), or anti-totall-H3 (ab1791, 1:2000), or anti-Myelin Basic Protein antibody [IGX3421] (ab209328, 1:1000). And second antibody was selected as follows: goat anti-rabbit (ab150077, 1:1000), or goat anti-rat (ab150117, 1:1000). Primary antibody was incubated overnight at 4°C and second antibody was incubated for 1 hour at room temperature.

Immunofluorescence staining

The rats were anesthetized by intraperitoneal injection of chloral hydrate, and the rats' hearts were exposed. The rats were perfused with 4% paraformaldehyde (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). After perfused and fixed, the brain tissue was taken by decollation and the brain tissue was placed in % paraformaldehyde solution, soaked overnight at 4°C or 12 h, and then dehydrated with 15% and 30% sucrose solution. Rat brain tissue was serially sliced at coronal position before and after the injection point, and the slice thickness was about 2 mm, then frozen in liquid nitrogen and stored in a -80°C refrigerator (750, Thermo Fisher, MA, USA). Continuous coronal slices were performed in a constant-humidity cryomicrotome with 6 μm thick. Slices were placed directly in slides and stored in low-temperature refrigerator at -80°C.

Brain tissue slice was taken and placed at room temperature for 20 min. After drying, immunohistochemical tissue range was labeled. It was washed with PBS (135 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8 mM K_2HPO_4, pH was adjusted with HCl and NaOH to 7.2) for 3 times/5 min, then goat serum (Hyclone, MA, USA) was used for blocking at 37°C for 1 h. After goat serum was removed, Anti-Myelin Basic Protein antibody [IGX3421] (ab209328, 1:200), or anti-CD16 (ab203883, 1:200) and anti-Iba (ab5076, 1:500), or anti-CD206 (ab64603, 1:500) and anti-Iba (ab5076, 1:500) were added and incubated overnight at 4°C. It was washed with PBS for 3 times/5 minutes at room temperature in the dark. Secondary antibodies [sheep anti-rabbit (ab150077, 1:1000), goat anti-rat (ab150117, 1:1000) or goat anti-chicken (ab150169, 1:1000)] were added and incubated at 37°C for 1 h. It was washed with PBS for 3 times/5 minutes at room temperature in the dark. After mounting, it was observed with fluorescence microscope, and images were collected. The above antibodies were purchased from Abcam. Using a Dapi Fluoromount-G blocker to block (Southern Biotech, USA), the cells were observed and photographed with a fluorescence microscope. The above antibodies were purchased from Abcam.

Statistical analysis

SPSS 20.0 was used for the statistical analysis of the data in this study. Data were expressed

as (mean ± standard deviation). Student's t-test was used to compare differences between two groups. One-way ANOVA was used to compare differences between different groups. P<0.05 indicated a significant difference.

Results

Scriptaid attenuated infarct volume and neurobehavioral dysfunction

7 days after HBID, we detected the infarct volume of rats in different group and found that HDAC inhibition scriptaid could significantly attenuated the infarct volume of rats (n = 7, P = 0.015). This showed that the intervention of scriptaid could reduce the infarct volume of brain tissue to a certain extent, and had certain protective effect on neonatal rat HIBD.

Rotarod test and wire hang test were used for neurobehavioral evaluation. As showed in **Figure 1**, Scriptaid improved the performance of HBID rat in the rotarod test (time = 5 and 7 days after HBID) (**Figure 1B**) and wire-hang test (time = 3, 5 and 7 days after HBID) (**Figure 1C**). The results of the neurobehavioral evaluation had showed that scriptaid could reduce nerve damage of HBID rat.

Morris water maze was an experimental device composed of a circular water tank and an automatic video analysis system. It could be used to detect the behavior and memory function of experimental animals, and could be used to indirectly assess neurobehavioral behavior. 7 days after HBID, we started the Morris water, and the results had showed that scriptaid improved the performance of HBID rat in the positioning navigation test (**Figure 1D**) and space exploration test (**Figure 1E**). The results of Morris water maze showed that the spatial learning and memory ability of rats after HBID was significantly reduced, but Scriptaid could improve them.

Scriptaid prevented neuronal degeneration

Increased enzymatic activity of histone deacetylase can result in decreased histone acetylation. Previous research had shown that HDAC participated in many diseases of the central nervous system including HBID. Can the use of the HDAC inhibitor scriptaid reverse this pathological process and thereby exert a neuroprotective effect? We injected HDAC inhibitor Scriptaid intraperitoneally immediately after HBID, and killed rats to take brain tissue at 7 days after HBID. We found that (**Figure 2A**) the expression of HDAC-1, HDAC-2 and HDAC-3 in HBID + Veh group were much more than that in the sham group, but Scriptaid could significantly inhibit the expression (P<0.05). More meaningful was that Scriptaid could significantly increase the acetylation of H3 and H4 in HBID rats. The results of these studies indicated that the expression of HDAC-1, HDAC-2 and HDAC-3 increased and the acetylation level of H3, H4 decreased after HBID, and HBID was exacerbated. But Scriptaid could significantly increase the level of histone acetylation by inhibiting the activity of HDAC after HBID.

Neurodegenerative change is a common aftereffect of HBID and usually results in irreversible damage to neurons. FluoroJadeC (FJC) is a fluorescent dye that combines with denatured neurons to glow green, whereas normal neurons do not glow green when combined with FJC. As shown in **Figure 2B**, neurons are almost nonregressive in the sham group, but a large number of neurons exhibited degenerative changes at 7 days after HBID in infarcted brain tissue of HBID + Veh group, and Scriptaid could significantly prevent neuronal degeneration. In addition, neuronal degenerative lesions often resulted in the loss of asymptotic structure and function of neurons. Microtubule-associated protein 2 (MAP2) is a phosphoprotein that regulates the stability of microtubules. It is mainly distributed in the cell bodies and dendrites of neurons and is considered to be a more specific marker of neuron dendrites. In our paper, we found that (**Figure 2B**, **2C**) the expression of MAP2 protein decreased in infarcted brain tissue of HBID + Veh group, and Scriptaid could significantly increase the expression of MAP2 protein.

Scriptaid protected oligodendrocytes indirectly through microglia

The main function of oligodendrocytes (OLG) is to surround the axons in the central nervous system, form an insulating myelin sheath structure, assist in the efficient and efficient transmission of bioelectric signals, and maintain and protect the normal functions of neurons. Demyelinating disorders of the central nervous system can also cause neuronal damage or

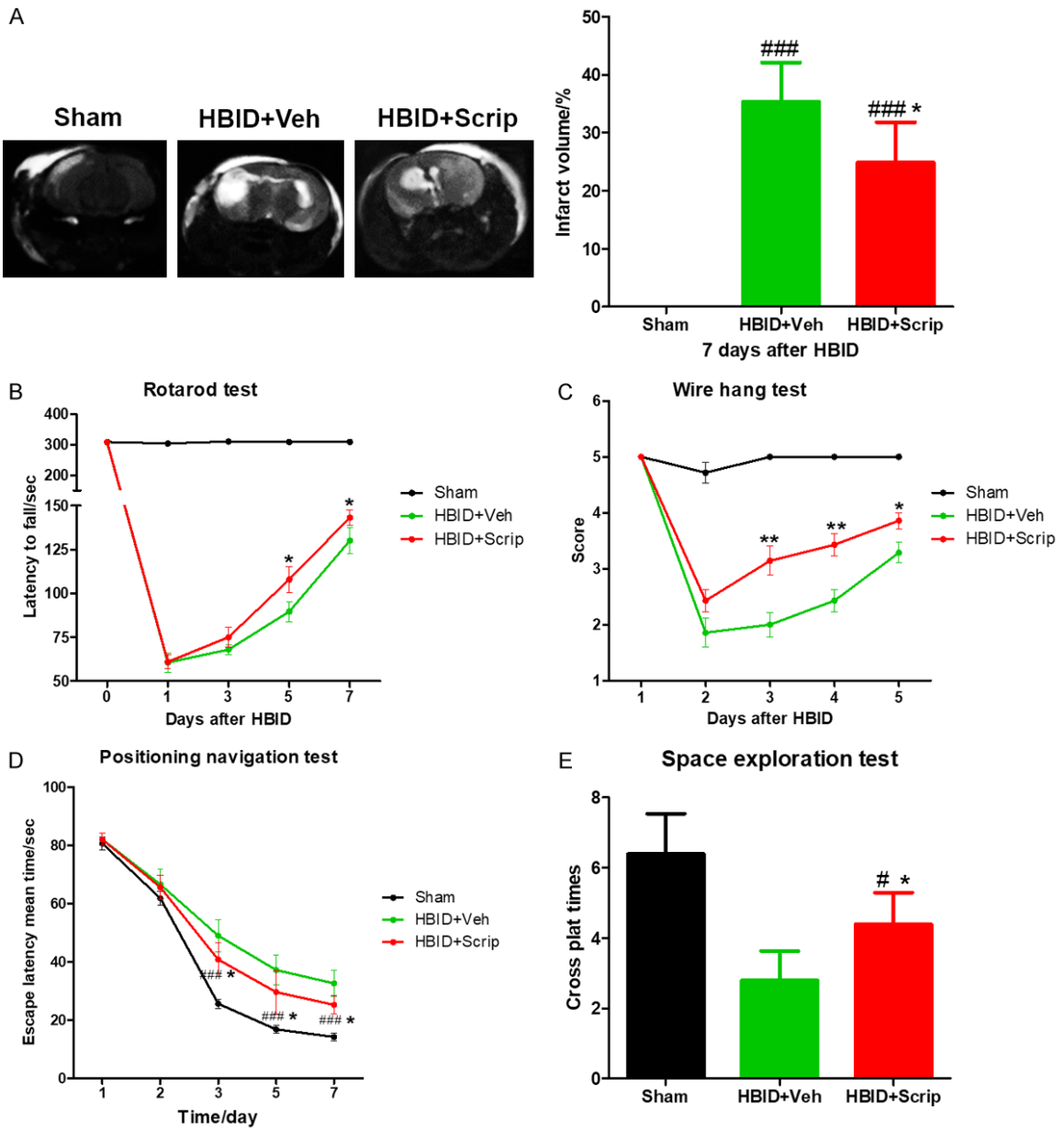


Figure 1. HDAC inhibition attenuated infarct volume and improved sensorimotor functions. (A) Infarct volume of rats at 7 days after HBID which was detected by MRI; (B-E) Scriptaid improved the performance of HBID rat in the rotarod test (B), wire-hang test (C), positioning navigation test (D) and space exploration test (E). There were 7 rats for each group in MRI, rotarod test and wire-hang test, 5 rats for each group in positioning navigation test and space exploration test. Data are shown as the mean ± SD; Compared with Sham group, # was P<0.05 and ### was P<0.001; Compared with HBID + Veh group, * was P<0.05 and ** was P<0.01.

mental illness. Sphingomyelin basic protein (MBP) is a characteristic protein of mature oligodendrocytes. We detected the expression of MBP protein in rat brain at 7 days after HBID by western blot, and found that (**Figure 3A**) the expression of MBP protein in HBID + Veh group was significantly less than that in sham group (P<0.05), but Scriptaid could significantly enhance its expression (P<0.05). This suggested that HDAC inhibition had a protective effect on oligodendrocytes after HBID in rats.

However, the experiments in vitro had found (**Figure 3B**), that Scriptaid had no significant effect on oligodendrocyte MBP protein expression after OGD. When we co-cultured with dif-

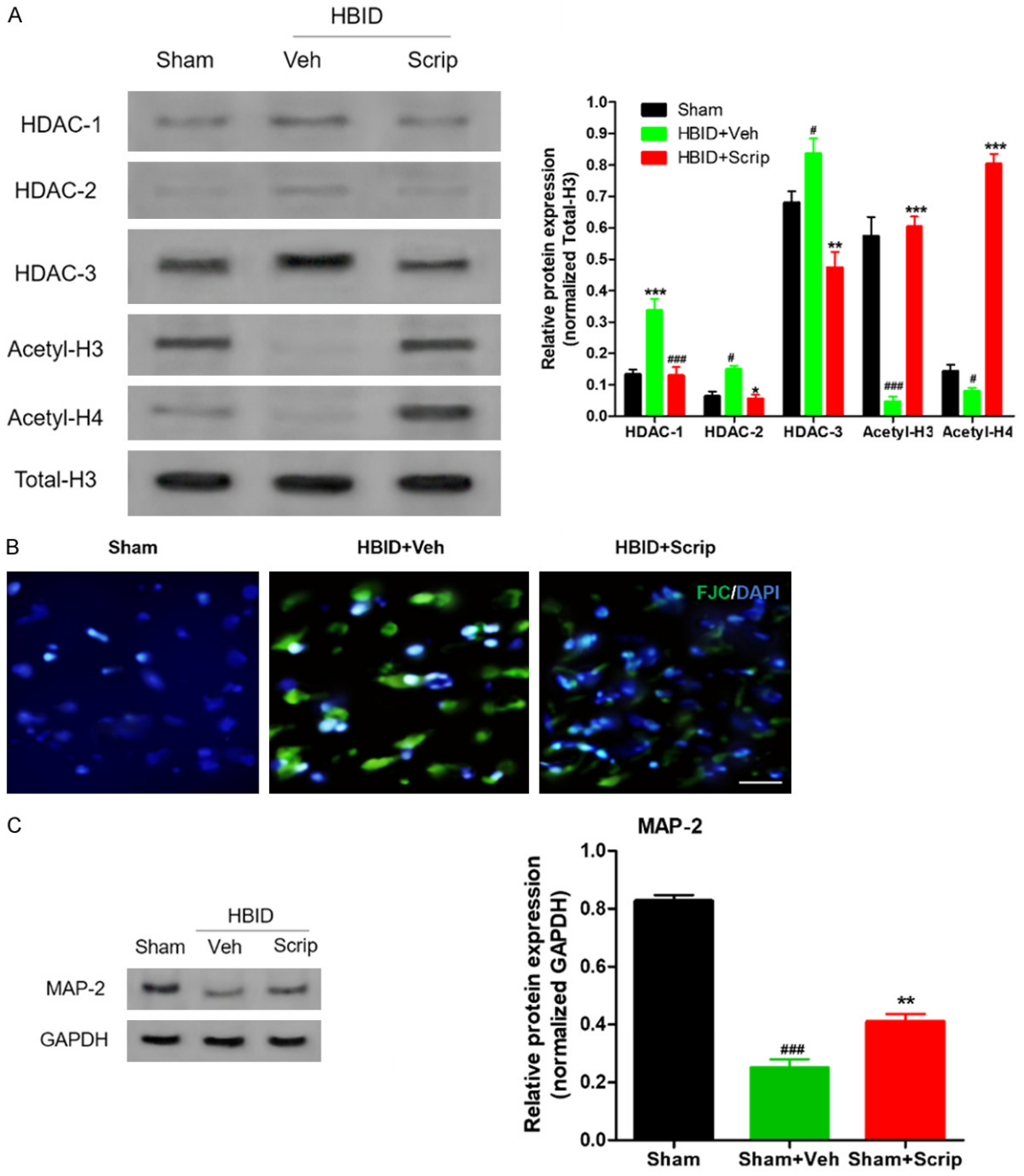
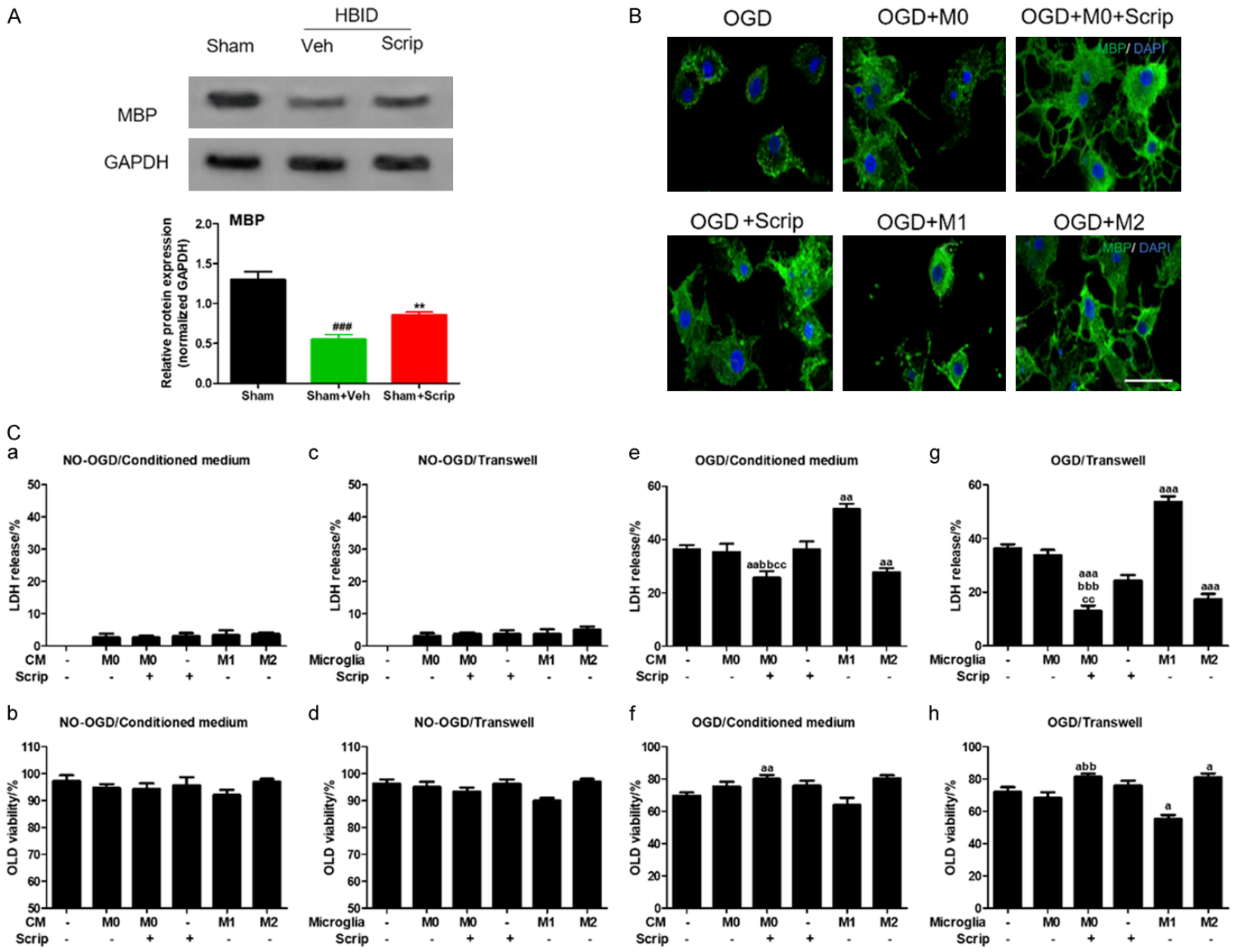


Figure 2. HDAC inhibition increases the level of histone acetylation and prevents neuronal degeneration. A. The expression of HDAC in nuclear protein extracts of the rats brain at 7 day after HBID (n = 7); B. Neuronal degeneration was evaluated by FJC staining at 7 day after HBID (n = 7) (25 um); C. The expression of MAP-2 protein in the rat brain tissue at 7 day after HBID (n = 7). Data were shown as the mean ± SD. Compared with Sham group, # was P<0.05, ## was P<0.05 and ### was P<0.001; Compared with HBID + Veh group, * was P<0.05, ** was P<0.01 and *** was P<0.001.

ferent induced phenotype microglia and OLG, Scriptaid had a significant effect on oligodendrocyte MBP protein expression after OGD. As shown in **Figure 3C**, Scriptaid-treated microglia cultures protected OLG after OGD, and this protection was more obvious in transwell system (**Figure 3Ce-Ch**). In addition, we also found that M1 phenotype microglia inhibited the activity of OLG cells after OGD, while M2 has a protective effect.


A
HBID
Sham
Veh
Scrip
MBP
GAPDH
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Relative protein expression (normalized GAPDH)
Sham
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B
OGD
OGD+M0
OGD+M0+Scrip
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OGD+M2
MBP/ DAPI
C
a NO-OGD/Conditioned medium
b NO-OGD/Conditioned medium
c NO-OGD/Transwell
d NO-OGD/Transwell
e OGD/Conditioned medium
f OGD/Conditioned medium
g OGD/Transwell
h OGD/Transwell
LDH release/%
OLD viability/%
CM
Microglia
Scrip
M0
M1
M2

Figure 3. HDAC inhibition protected oligodendrocytes indirectly through microglia. A. The expression of MBP protein in rat brain tissue at 7 day after HBID; B. MBP and DAPI staining of oligodendrocytes exposed to OGD and cultured with CM from microglia exhibiting M0 (with Scriptaid or vehicle), M1, or M2 phenotypes; C. Cell death was detected by LDH release and the viability of OLD was detected by MTT assay. Data are shown as the mean ± SD; Compared with Sham group, ### was P<0.001; Compared with HBID + Veh group, ** was P<0.01; a was P<0.05, aa was P<0.01 and aaa was P<0.001 vs the first pillar in Ca-Ch); b was P<0.05, bb was P<0.01 and bbb was P<0.001 vs the second pillar in Ca-Ch); c was P<0.05, cc was P<0.01 and ccc was P<0.001 vs the fourth pillar in Ca-Ch).

The above experiments showed that the protective effect of HDAC inhibition on OLG after HBID in rats was indirect, which may be through the inhibition of the proinflammatory effect of microglia to achieve the OLG protective effect after HBID [21].

Scriptaid promoted microglia to the M2 phenotype after HBID

Our in vivo and vitro results demonstrated that Scriptaid prevented neuronal degeneration and the death of OLG cells after HBID, and these results may be due to Scriptaid's inhibition of the pro-inflammatory effects of microglia. In order to verify this conclusion in vivo, we measured the number of CD16-marked M1-type microglia and CD206-marked M2-type microglial cells in cerebral infarct tissue at 7 days after HBID by immunofluorescence (**Figure 4A**), and detected the expression of CD16 and CD206 mRNA by RT-qPCR. We found that the numbers of M1 and M2 microglia in infarcted brain tissue of HBID + Veh rats were much more than that in sham group (P<0.05), and Scriptaid could reduce the number of M1 microglia and increase M2 microglia at 7 days after HBID (**Figure 4B**). The same trend was also reflected in the expression of CD16 and CD206 mRNA (**Figure 4C**).

In addition, we also found that mRNA of IFN-γ, TNF-α, IL-4, and IL-13 in cerebral infarction of HBID + Veh rats after HBID7 days was significantly higher than that in the sham group, and Scriptaid could inhibit the IFN-γ and TNF-α expression and increase of IL-4 and IL-13 mRNA expression. This suggested that HDAC inhibition could promote micropolarization of microglia into M2 microglia, and inhibited inflammatory responses.

Discussion

At present, there are no therapies or drugs that can cure the cognitive and motor deficits in HBID patients [22, 23]. Another need to clarifyis that the degree of brain development in 7-day-old rats is basically the same as that of full-term newborns [24]. Therefore, in this study, we use 7-day-old SD suckling rats to make HBID model. After 7 days of Scriptaid treatment, we found that Scriptaid could reduce the infarct volume of brain tissue to a certain extent, and had certain protective effect on neonatal rat HIBD.

Scriptaid is a HDAC inhibitor, and histone acetylation is an important mechanism of gene transcription regulation and is mainly regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs) [25, 26]. HATs can activate certain genes and promote transcription, but HDACs inhibit transcription by inhibiting the binding of gene promoters to transcriptional regulatory elements [10]. With the deepening of epigenetic studies, it had been found that histone deacetylase was closely related to the occurrence and development of tumors, nervous system diseases, and immune system diseases, so HDACs and their inhibitors HDACIs had attracted more and more attention and became a hot topic in the design and synthesis of new drugs [27]. Recently, a large number of HDACIs have been designed and synthesized for use in animal models of various neurodegenerative diseases [28]. Guohua Wang, et al. [13] had found that HDAC inhibitor Scriptaid could prevent white matter injury after traumatic brain injury. Previous work found [29, 30] that HDAC inhibitors played an important role in multiple aspects of ischemic stroke injury mechanisms to reduce brain tissue damage and promote neuronal plasticity and functional recovery after ischemia by regulating histones and non-histone modifications, upregulate the expression of genes and proteins with neuroprotective functions and inhibit the expression of harmful genes and proteins [14, 31].

In this paper, we found Scriptaid not only could increase the corresponding histone acetylation level significantly by inhibiting the activity of

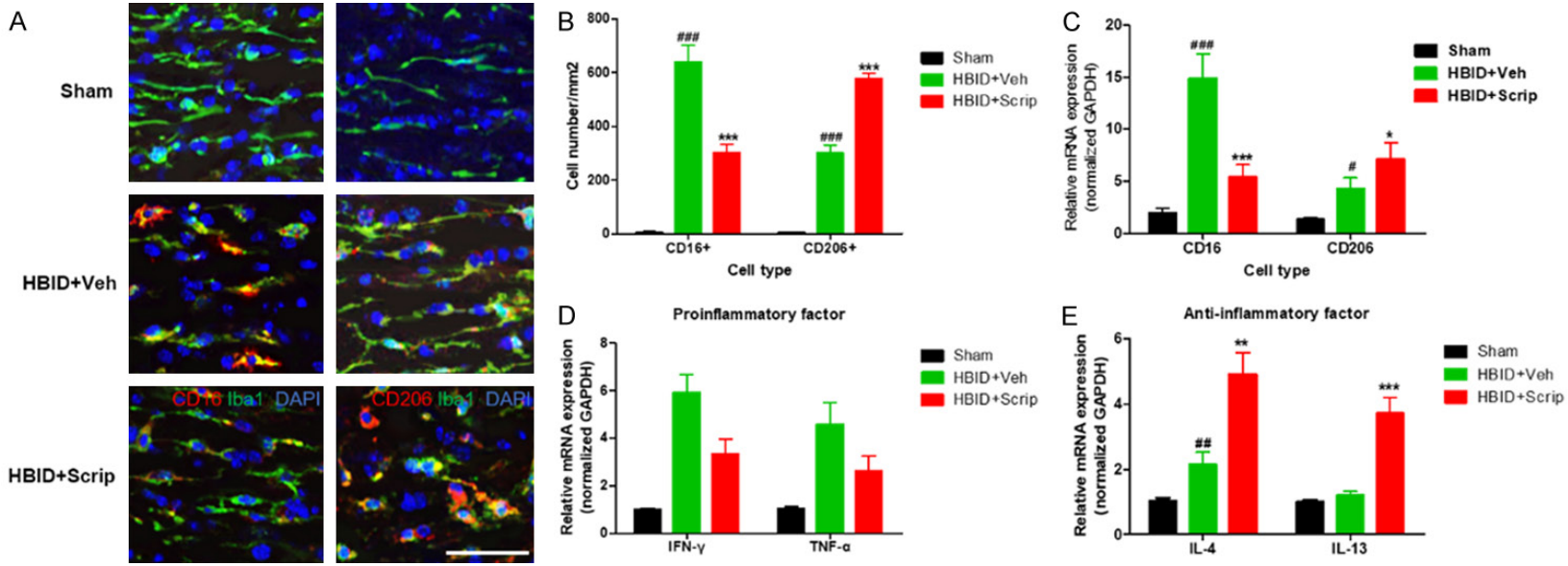

Figure 4. HDAC inhibition reduced M1 microglia and increase M2 microglia in cerebral infarction of HBID rats at 7 days after HBID (n = 7). (A, B) Immunofluorescence was used to detect M1 (marked by CD16) and M2 (marked by CD206) microglia in cerebral infarction of HBID rats. Red fluorescence was CD16/CD206, green was Iba1, and blue was DAPI. (C-E) RT-qPCR was used to detect the mRNA expression of CD16/CD206 (C), proinflammatory (D) and factor anti-inflammatory (E) in cerebral infarction of HBID rats. Compared with Sham group, # was P<0.05, ## was P<0.01, and ### was P<0.001; Compared with HBID + Veh group, * was P<0.05, ** was P<0.01, and *** was P<0.001.

HDAC in brain tissue of HBID model mice, but also could reduce the number of degenerative neurons in cerebral infarction. However, the experiments in vitro had found (**Figure 3B**), Scriptaid had no significant effect on oligodendrocyte MBP protein expression after OGD. When we co-cultured with different induced phenotype microglia and OLG, Scriptaid had a significant effect on oligodendrocyte MBP protein expression after OGD. This result is consistent with the experimental results of Guohua Wang et al [13], and suggest that Scriptaid's protective effect on neurons after HBID is indirect.

Microglial/macrophages are the most important regulatory cells in the process of innate immune responses in central nervous system injury and disease [20, 32]. The role of microglia in the central nervous system after injury is currently receiving widespread attention. Recent research results strongly supported the idea that microglia had both good and bad roles, and that these immune cells migrated to M1 or M2 subunits through different activation pathways [33, 34]. On the other hand, the activation of microglia/macrophages can remove cell debris, remodeled tissue integrity and thus facilitating the protection of brain damage. However, a large number of studies had also shown that microglial released pro-inflammatory factors that could promote neurological dysfunction and cell death [35, 36]. That means microglia could produce pro-inflammatory cytokines to promote cell damage, but at the same time could remove necrotic tissue to participate in the regeneration process [37, 38]. Our in vitro results demonstrated that M1 microglia inhibited OLG activity after OGD, and M2 microglia enhanced OLG activity after OGD. In vivo it was demonstrated that HDAC inhibition by Scriptaid could promote micropolarization of microglia into M2 microglia, and inhibited inflammatory responses. Here are two points we need to clarify with the reader. First, the neuroinflammation of the central nervous system is mainly mediated by microglia and giant salivary cells, and these two cells participate in mediating secondary cascade damage after brain injury [39, 40]. Second, acute inflammation can exert many protective effects [20], but chronic inflammation can worsen the damage which is consistent with the traditional view of chronic inflammation [41, 42]. In this paper, we chosen to sacrifice rats for study on the 7th day after HBID was aimed to observe the chronic inflammatory response of HBID rats after HBID by Scriptaid. We found Scriptaid could promote micropolarization of microglia into M2 microglia, and inhibited inflammatory responses after HBID.

Conclusions

HDAC inhibition by Scriptaid inhibits inflammatory responses and protects brain by promoting the polarization of microglia in brain tissue to M2 microglia after HBID. All in all, our research provided a new possibility for the treatment of HBID.

Disclosure of conflict of interest

None.

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