The neuroprotection of hyperbaric oxygen therapy against traumatic brain injury via NF-κB/MAPKs-CXCL1 signaling pathways

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Abstract
It is well known that hyperbaric oxygen (HBO) therapy achieves neuroprotective effects by modulating neuroinflammatory responses. However, its underlying therapeutic mechanisms are not yet fully elucidated. Based on our previous studies, we further investigated whether HBO therapy exerts neuroprotective effects in vivo by regulating the nuclear factor-kappa B (NF-κB)/mitogen-activated protein kinases (MAPKs) chemokine (C-X-C motif) ligand (CXCL)1 inflammatory pathway. In our study, a rat model of traumatic brain injury (TBI) was established by controlled cortical impact (CCI) to verify that the expression of CXCL1 and chemokine (C-X-C motif) receptor (CXCR)2 increased after TBI, and CXCL1 was mainly expressed in astrocytes, while CXCR2 was mainly expressed in neurons. Increased apoptosis of cortical nerve cells in the injured cortex was also found after TBI. Reduced nerve cell apoptosis with improved neurological function was observed after application of a CXCR2 antagonist. The expression of phospho-extracellular signal-regulated kinase (p-ERK), phospho-c-Jun N-terminal kinase (p-JNK) and p-NF-κB increased after TBI, and application of ERK, JNK and NF-κB inhibitors decreased expression of CXCL1 and CXCR2 in rats. We further found that HBO therapy down-regulated the expression of p-ERK, p-JNK, p-NF-κB, CXCL1, and CXCR2, and reduced nerve cell apoptosis, improved the neurological function of TBI rats, and ultimately alleviated the secondary injury. In conclusion, HBO therapy may exert neuroprotective effect by regulating the NF-κB/MAPKs (JNK and ERK)-CXCL1 inflammatory pathways following TBI, which probably provide the theoretical and experimental basis for the clinical application of HBO therapy in the treatment of TBI.

Keywords
Hyperbaric oxygen · Traumatic brain injury · CXCL1 · CXCR2

Abbreviations
TBI · Traumatic brain injury
CCI · Controlled cortical impact
HBO · Hyperbaric oxygen

CXCL · Chemokine (C-X-C motif) ligand
CXCR · Chemokine (C-X-C motif) receptor
CCL · Chemokine (C-C motif) ligand
CCR · Chemokine (C–C motif) receptor
MAPKs · Mitogen-activated protein kinases
NF-κB · Nuclear factor-kappa B
JNK · C-Jun N-terminal kinase
ERK · Extracellular signal-regulated kinase
GFAP · Glial fibrillary acidic protein
IBA-1 · Ionized calcium binding adapter molecule 1
NeuN · Neuronal nuclei
TUNEL · Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling
DAPI · 6-Diamidino-2-phenylindole
DMSO · Dimethyl sulfoxide
RT-qPCR · Real-time fluorescence quantitative PCR
IL · Interleukin
TNF-α · Tumor necrosis factor-α
p- · Phospho-
mNSS · Modified neurological severity scores

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**Introduction**

The yearly incidence of traumatic brain injury (TBI) is estimated at 50 million cases worldwide, and thus TBI is a major global health challenge (Khellaf et al. 2019). The high rates of TBI-associated morbidity and mortality in China, a large developing country, has become a major public health concern (Jiang et al. 2019). TBI includes primary and secondary injuries. Compared to primary injuries, secondary injuries last longer and are the focus of scientific research and clinical treatment for TBI. Neuroinflammation is a major pathogenic mechanism leading to secondary brain injuries following TBI. It has been reported in the literature that the expression of various inflammatory factors, such as interleukin (IL)-1β, chemokine C–C motif ligand (CCL)2, Chemokine (C-X-C motif) ligand (CXCL)1, interferon-γ, tumor necrosis factor-α (TNF-α), IL-6, IL-1α, IL-10 and IL-8, is altered after TBI. These factors, involved in the molecular regulatory mechanisms of neuroinflammatory response, are classified into two major groups: pro-inflammatory and anti-inflammatory factors (Dalgard et al. 2012; Deng et al. 2020; Di Battista et al. 2016; Kumar et al. 2017). Moreover, it has been shown that neuroinflammation modulation may be a favorable option for the alleviation of secondary brain injuries and to ameliorate the outcome of TBI (Chen et al. 2019; Finnie. 2013; Morganti-Kossmann et al. 2019).

The use of hyperbaric oxygen (HBO) for the treatment of TBI has been controversial, mainly because of uncertainty of its efficacy, complications associated with HBO therapy, and the fact that the therapeutic mechanisms have not been completely clarified (He et al. 2019; Hu et al. 2016; Wee et al. 2015; Wolf et al. 2012). Numerous animal studies and clinical practice have demonstrated that HBO treatment can reduce secondary injury after TBI. In 2018, a neurosurgery clinical team from the University of Minnesota published a review on the treatment of acute TBI with HBO that collected and analyzed 30 clinical and animal research articles. Their results showed clear efficacy of HBO in the treatment of acute TBI (Daly et al. 2018). A comprehensive search indicated that HBO might be beneficial to moderate-to-severe TBI as a relatively safe adjunctive therapy if feasible (Crawford et al. 2017). HBO treatment can reduce secondary injury after TBI, and its mechanism of action may be related to the reduction of cerebral edema, inhibition of astrocyte proliferation, alleviation of apoptosis of nerve cells, improvement of brain metabolism, and modulation of neuroinflammation (He et al. 2019; Daly et al. 2018; Liu et al. 2015, 2018, 2013a; Xing et al. 2018; Liang et al. 2020).

It has been reported in the literature that HBO treatment achieves neuroprotective effects by modulating neuroinflammatory responses following TBI, and the potential mechanism may be related to the altered expression of various inflammatory factors such as IL-1β, IL-6, CCL2, IL-10, and TNF-α (Wee et al. 2015; Chen et al. 2014; Meng et al. 2016; Zhang et al. 2014). The literature and our previous study have found that the expression of several chemokines and their effector receptors are increased after TBI and promote neuroinflammation by regulating neuronal–glial interactions, and HBO can modulate activation of astrocytes and microglia (Dalgard et al. 2012; Liu et al. 2013a,b; Jassam et al. 2017; Baratz-Goldstein et al. 2017; Lim et al. 2013). Through in vitro studies of primary cultured astrocytes, our group found that HBO therapy can downregulate the expression of chemokines CXCL1 and CCL2 and affect inflammatory responses by inhibiting the lipopolysaccharide (LPS)-induced nuclear factor-kappa B (NF-κB)/mitogen-activated protein kinases (MAPKs, JNK and ERK)-CCL2/CXCL1 inflammatory pathway (Liu et al. 2018). In the present study, we further examined whether HBO therapy exerts neuroprotective effects by modulating the NF-κB/MAPKs-CXCL1 inflammatory pathway through in vivo studies.

**Materials and methods**

**Animals and surgery**

The rat TBI model was established by the cortical controlled injury (CCI) method described previously (Beretta et al. 2017; Romine et al. 2014). All experimental treatments were in accordance with the Chinese “Guidelines for the Care and Use of Laboratory Animals”. Healthy adult male Sprague–Dawley rats (n = 284) weighing 230–250 g were purchased from the Experimental Animal Center of Nantong University (Nantong, China). All rats were placed in an environment with constant temperature and humidity and could drink and eat freely in a 12-h day and night cycle. After intraperitoneal anesthesia with 0.4 mL/100 g chloral hydrate, the rat's head was fixed on a stereotaxic device, a hole with a diameter of 6 mm was drilled in the right parietal bone (3.0 mm behind bregma and 3.0 mm centered on the right side of the sagittal suture), the skull was lifted to expose the dura mater, and a pneumatic impact device (TBI 0310, Precision Systems and Instrumentation, USA) was employed to severe model TBI. The impact parameters of velocity were 4.0 m/s, depth, 3.0 mm, and dwell time, 150 ms (Chiu et al. 2016; Xiong et al. 2013). The sham group underwent the same craniotomy, but without impingement.

| Acronym  | Description                      |
|----------|----------------------------------|
| MWM      | Morris water maze               |
| PBS      | Phosphate buffer saline          |
| BSA      | Bovine serum albumin            |
Modified neurological severity scores (mNSS)

The mNSS method was used to evaluate neurological function (Lu et al. 2007). Briefly, the aspects of the test included motor (6 points), sensory (2 points), balance beam test (6 points), lack of reflexes and abnormal activity (4 points). If the animal did not complete the required movements, one point was awarded (normal score, 0; maximal deficit score, 18). The higher the total score obtained by the animal, the more severe the nerve damage.

Drug treatment

SD male rats were randomly divided into five groups: sham, TBI, TBI vehicle, TBI low-dose, and TBI high dose. The chemokine (C-X-C motif) receptor 2 (CXCR2) antagonist SB225002 was purchased from Tocris (Bristol, UK). The extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and NF-κB inhibitors, PD98059, SP600125, and BAY117082, respectively, were purchased from Calbiochem (Merck, Darmstadt, Germany). The antagonist and the three inhibitors were adjusted dissolved in 1 μL 1% dimethyl sulfoxide (DMSO) and diluted with 9 μL 0.01 mmol/L phosphate buffer saline (PBS) to a low dose of 2.5 μg/10 μL and a high dose of 25 μg/10 μL, respectively (Guan et al. 2005; Jalilzad et al. 2019; Lin et al. 2014; Manjavachi et al. 2010). The TBI + vehicle group was treated with 1 μL 1% DMSO and 9 μL 0.01 mmol/L PBS. As described previously (Liu et al. 2013b), mini-osmotic pumps (ALZET 1003D, Cupertino, CA, USA) were used to administer drugs. At 4 h after TBI, the rats were re-anesthetized and the pumps were implanted to infused vehicle, low-dose, or high-dose drugs for 3 days running at 0.5 μL/h.

HBO therapy

SD male rats were randomly divided into four groups: sham, sham + HBO, TBI, and TBI + HBO. HBO therapy was given 6 h after injury and rats in the sham + HBO group and TBI + HBO group were subjected to continuously HBO therapy once a day for 3 days (Liu et al. 2013a). The rats were placed in a hyperbaric chamber and the pressure in the chamber was slowly increased to 0.2 MPa for about 15 min, then maintained at 0.2 MPa for about 60 min, and finally slowly decreased to atmospheric pressure for 15 min before moving the rats out of the hyperbaric chamber. During treatment, the oxygen concentration in the chamber was kept above 95%. The sham and TBI group were exposed to the chamber without pressure.

Immunofluorescent staining

After rats were intraperitoneal anesthetized and rapidly perfused with 4% paraformaldehyde, brain tissues were removed and placed in 4% paraformaldehyde at 4 °C overnight for post-fixation. The brain tissues were placed in 20% sucrose for dehydration for 2 days and then replaced with 30% sucrose for dehydration for another 2 days. The brain tissues were cryosectioned to a thickness of 20 μm and further immune-stained with fluorescence. Briefly, sections were incubated with 1% bovine serum albumin (BSA) at room temperature, and next incubated overnight at 4 °C with the following antibodies: CXCL1 antibody (A00533, rabbit, 1:50, Boster, Wuhan, Hubei, China), CXCR2 antibody (BA0732-2, rabbit, 1:50, Boster, Wuhan, Hubei, China), glial fibrillary acidic protein (GFAP) antibody (astrocyte marker, MAB360, mouse, 1:1000, Millipore, Billerica, MA, USA), ionized calcium binding adapter molecule 1 (IBA-1) antibody (microglial cell marker, ab5076, goat, 1:500, abcam, Boston, USA) and neuronal nuclei (NeuN) antibody (neuron marker, MAB377, mouse, 1:1,000, Millipore, Billerica, MA, USA). The next day, the sections were incubated with Cy3-linked secondary antibody or Alexa 488-linked secondary antibody (1:100, Jackson ImmunoResearch, West Grove, PA) for 2 h at room temperature. The stained sections were rinsed three times on a shaker for 15 min each, then air dried and sealed on slides. The stained slides were observed by a Nikon fluorescence microscope and images were captured by CCD Spot. For each antibody, three consecutive sections from central injured area (3 mm behind bregma) per animal were used for staining. Double-blind method was used to count the number of target cells from three microscopic fields selected randomly in the injured cortex in each section. The percent of cells was the number of co-labeled cells divided by the number of target cells.

Real-time fluorescence quantitative PCR (RT-qPCR)

Total RNA was extracted from the cerebral cortex of the injured area using Trizol reagent (Invitrogen, Carlsbad, California, USA). Total RNA (1 μg) was reverse transcribed into cDNA according to the manufacturer’s instructions (Takara, Table 1

| Genes | Primers | Sequences |
|-------|---------|-----------|
| GAPDH | Forward 5'-TCTACCCCAATGTACCCG-3' |
|       | Reverse 5'-CTTTTATGGCCCTCGG-3' |
| CXCL1 | Forward 5'-GCACCAAACCGAGTCATA-3' |
|       | Reverse 5'-GGGGACACCTTTAGCATTCT-3' |
| CXCR2 | Forward 5'-TGGTCTGTGTCTGCCTCCTCTCG-3' |
|       | Reverse 5'-CGTTCTGGCGTTCAGGTCTC-3' |
Shiga, Japan). After addition of SYBR green I, RT-qPCR analysis was performed in a real-time detection system (Rotor-Gene 6000, Hamburg, Germany). The primers used are listed in Table 1. The RT-qPCR amplification program was as follows: first pre-denaturation at 95 °C for 3 min, followed by 40 thermal cycles at 95 °C for 10 s, 60 °C for 30 s, and finally 95 °C for 15 s, 60 °C for 60 s, and 95 °C for 15 s to generate the melting curve. The melting curves were used to ensure that there were no nonspecific products. Quantitative analysis was performed by the $2^{-\Delta\Delta CT}$ method.

**Western blot analysis**

The tissue from the damaged area of the cerebral cortex was added to 1.5 mL EP tubes containing 250 μL protein lysate and homogenized. The lysis reaction proceeded for 30 min, and the supernatant was collected by centrifugation. The total protein concentration was measured by BCA protein assay (Pierce, Rockford, IL, USA). Protein samples (30 μg) were separated on 10% SDS-PAGE gels and transferred to PVDF membranes. The membranes were blocked with 5% BSA for 2 h at room temperature to avoid non-specific binding. Following incubation with antibodies, such as p-ERK (9101, rabbit, 1:1000, Cell Signaling, Boston, USA), p-JNK (4688, rabbit, 1:1000, Cell signaling, Boston, USA), p-NF-κB (3033, rabbit, 1:1000, Cell signaling, Boston, USA), or GAPDH antibody (MAB374, mouse, 1:10,000, Millipore, Billerica, MA, USA), at 4 °C overnight, the membranes were further incubated with IRDye 800CW antibody for 2 h in the dark at room temperature. Images were captured using the Odyssey Imaging System (LI-COR Bioscience, Lincoln, NE), and grayscale values were analyzed using Image J software (NIH, Bethesda, MD, USA).

**TUNEL staining**

Samples for TUNEL staining were prepared in the same way as described for immunofluorescence double staining. Apoptosis was detected by terminal deoxynucleotidyl transferase-mediated dUTP nick 3’ end labeling (TUNEL) using an apoptosis detection kit (Vazyme, Nanjing, China), according to the manufacturer’s instructions. After completion of apoptosis staining, nuclei were stained with 6-diamidino-2-phenylindole (DAPI). The stained slides were examined with a Nikon fluorescence microscope and images were obtained with a CCD Spot camera. TUNEL-positive cells in the cerebral cortex of the injured area were counted in each slide using Image J software (NIH, Bethesda, MD, USA). Five animals in each group were used. Three consecutive sections from injured area (3 mm behind bregma) per animal were used for statistics. Three microscopic fields were selected randomly in the injured cortex in each section. Double-blind method was used to count the number of positive cells from the microscopic fields.

**Statistical analysis**

Experiments were conducted with 3–8 samples in each group. All data were expressed as MEAN ± SEM. Image J was used to count the number of TUNEL-positive cells and immunofluorescence double-label staining. For western blotting, ImageJ was used to measure the grayscale values of specific bands. The relative expression levels of p-ERK, p-JNK and p-NF-κB were standardized to the level of GAPDH. Multi-group comparisons were performed using one-way ANOVA with post hoc Bonferroni correction. All data were analyzed using GraphPad Prism 8.0 (San Diego, CA, USA).

**Results**

**CXCL1 was predominantly expressed in astrocytes of the injured cortex following TBI**

To visualize the cellular localization of CXCL1 in the cortex, we performed immunofluorescence double staining of CXCL1 with the astrocyte marker GFAP, the neuronal marker NeuN, and the microglia marker IBA-1, respectively. As shown in Fig. 1, about 69% of CXCL1 was co-labeled with GFAP, 30% with NeuN, and about 1% with IBA-1. The results suggested that CXCL1 was mainly expressed in astrocytes in the injured cortex after TBI.

**CXCR2 was mainly expressed in neurons of the injured cortex following TBI**

To confirm the cellular localization of CXCR2 in the cortex, we employed immunofluorescence double staining of CXCR2 with the cellular markers GFAP, NeuN, and IBA-1, respectively. Figure 2 shows that about 70% of CXCR2 was co-labeled with the neuronal marker NeuN, 28% with the microglial marker IBA-1, and 2% with the astrocyte marker GFAP. The results indicated that CXCR2 was mainly expressed in neurons in the injured cortex after TBI.

**CXCL1 and CXCR2 mRNA expression upregulated in the injured cortex of TBI rats**

The expression level of CXCL1 and CXCR2 mRNA were measured by RT-qPCR, respectively, in the peri-injured cortex at 1, 3, 7, and 10 days after TBI. As shown in Fig. 3, the mRNA expression levels of CXCL1 and CXCR2 peaked on the first day and then decreased compared with the sham group.
p-ERK, p-JNK, and p-NF-κB expression upregulated in the injured cortex of TBI rats

The expression trends of p-ERK, p-JNK, and p-NF-κB in the cortex of the injured area were assessed by western Blot at 1, 3, 7, and 10 days after TBI. As shown in Fig. 4, compared with the sham group, p-ERK and p-JNK peaked on the third day after TBI, whereas p-NF-κB showed a decreasing trend after peaking on the first day after TBI.

TBI-induced apoptosis of cortical nerve cells in the injured cortex in rats

TUNEL staining was employed to detect apoptosis at 1, 3, and 7 days after TBI. Figure 5 shows that the number of
TUNEL-positive cells increased at 1, 3, and 7 days after TBI compared with the sham group and showed a decreasing trend after reaching a peak on the first day.

**CXCR2 antagonist improved neurological function of TBI Rats**

To evaluate the effect of CXCL1-CXCR2 on the neurological function of TBI rats, mNSS was scored after cortical injection of CXCR2 antagonist SB225002 in the injured area of TBI rats. As shown in Fig. 6, the mNSS of rats after TBI was significantly higher than that of the sham group with impaired neurological function. The mNSS score of the TBI + high dose group was lower than that of the TBI + vehicle group after 3 days local injection of CXCR2 antagonist in the brain injury area, indicating that down-regulation of CXCR2 expression improves neurological function.
CXCR2 antagonist reduced nerve cell apoptosis in TBI rats

To evaluate the effect of CXCR2 antagonist SB225002 on the apoptosis of cortical nerve cells in the brain injury area of TBI rats, we performed TUNEL staining. As shown in Fig. 7, the number of TUNEL-positive cells in the cerebral cortex of TBI + high dose rats was significantly reduced compared with that in the TBI + vehicle group on day 3 post-TBI.

ERK, JNK, and NF-κB inhibitors downregulated expression of CXCL1 and CXCR2 mRNA in TBI rats

To verify whether ERK, JNK, and NF-κB regulate the expression of CXCL1/CXCR2, the expression changes of CXCL1/CXCR2 were observed after application of their inhibitors PD98059, SP600125, and BAY117082, respectively. As shown in Fig. 8, the mRNA expression of CXCL1...
Fig. 7 CXCR2 antagonist (SB225002) reduced nerve cell apoptosis in TBI rats on day 3 post-injury. A TUNEL-positive cells in the cortex of sham group. B–E TUNEL-positive cells in the injured cortex at 3 days in TBI, TBI vehicle, and TBI low-dose group, and TBI high dose group. F The number of TUNEL-positive cells in the injured cortex of TBI rats significantly reduced after application of high dose of CXCR2 antagonist. Values are expressed as mean ± SEM (n = 5/group). ***p < 0.001, vs. TBI vehicle group; ###p < 0.001, vs. sham group.

Fig. 8 ERK, JNK, and NF-κB inhibitors PD98059, SP600125, and BAY117082 down-regulated CXCL1 and CXCR2 mRNA expression on day 3 post-TBI. A CXCL1 mRNA expression decreased significantly at 3 days after continuous injection of high doses of ERK, JNK, and NF-κB inhibitors. B CXCR2 mRNA expression decreased significantly at 3 days after continuous injection of high doses of ERK, JNK, and NF-κB inhibitors. Values are expressed as mean ± SEM (n = 6/group). ***p < 0.001, **p < 0.01, *p < 0.05, vs. TBI vehicle group; ###p < 0.001, ##p < 0.01, vs. sham group.
and CXCR2 decreased significantly after 3 days of continuous injection of high doses of ERK, JNK, and NF-κB inhibitors compared with the TBI + vehicle group, indicating that ERK, JNK, and NF-κB could regulate CXCL1 and CXCR2.

**HBO treatment improved the neurological function of rats following TBI**

The effect of HBO therapy on the neurological function of TBI was observed by mNSS score. We found that the mNSS scores of the TBI + HBO group were significantly lower than that of the TBI group at 3, 7, 10 days after HBO treatment, shown in Fig. 9. These results showed that HBO treatment could improve the neurological function of rats after TBI.

**HBO treatment reduced nerve cell apoptosis in rats following TBI**

We evaluated the effect of HBO treatment on nerve cell apoptosis in the cerebral cortex of rats with TBI. In Fig. 10, the results showed that the number of TUNEL-positive cells in the cerebral cortex of the TBI + HBO group was significantly reduced compared with the TBI group on 1 and 3 days, indicating that HBO treatment could inhibit apoptosis of nerve cells in TBI rats.

**HBO treatment downregulated CXCL1 and CXCR2 mRNAs expression after TBI in rats**

To verify whether HBO treatment regulates expression of CXCL1/CXCR2, the mRNA of CXCL1 and CXCR2 were detected by RT-qPCR, respectively, after 1, 3, 7, 10 days of continuous HBO therapy. In Fig. 11, the results showed that the mRNA of CXCL1 and CXCR2 were significantly decreased compared with the TBI group at 1, 3 and 7 days, indicating that HBO treatment could down-regulate the expression of CXCL1 and CXCR2.

**HBO treatment down-regulated the expression of p-ERK, p-JNK, and p-NF-κB after TBI in rats**

To verify whether HBO therapy regulates CXCL1/CXCR2 expression by modifying ERK, JNK, and NF-κB, we tested the expression level of p-ERK, p-JNK, and p-NF-κB

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**Fig. 9** HBO treatment improved the neurological function of TBI rats. A mNSS scores of TBI + HBO group were lower than TBI group at 1 day, but the difference was not significant statistically. B–D mNSS scores of TBI + HBO group were significantly lower than TBI group at 3, 7, 10 days following HBO treatment. Values are expressed as mean ± SEM (n = 8/group). **p < 0.01, vs. TBI group**
Fig. 10 HBO treatment reduced nerve cell apoptosis of injured cortex. A–D TUNEL-positive cells at 1 day in the cortex of sham, sham + HBO, TBI and TBI + HBO group. E–H TUNEL-positive cells in the injured cortex at 3 days in sham, sham + HBO, TBI, TBI + HBO group. I–L TUNEL-positive cells at 7 days in the cortex of sham, sham + HBO, TBI and TBI + HBO group. M–O The number of TUNEL-positive cells at 1 and 3 days in the injured cortex with HBO treatment significantly reduced compared to TBI group. Values are expressed as mean ± SEM (Bar = 20 μm) (n = 5/group). **p < 0.01, *p < 0.05, vs. TBI group

Fig. 11 HBO treatment down-regulated CXCL1 and CXCR2 mRNAs expression following TBI. A–D HBO treatment down-regulated CXCL1 mRNAs expression following TBI at 1, 3 and 7 days. E–H HBO treatment down-regulated CXCR2 mRNAs expression following TBI at 1, 3 and 7 days. Values are expressed as mean ± SEM (n = 6/group). *** p < 0.001, ** p < 0.01, * p < 0.05, vs. TBI group
proteins by western blot after 3 days of continuous HBO treatment. In Fig. 12, the results showed that the levels of p-ERK, p-JNK, and p-NF-κB were significantly lower in the TBI + HBO group compared with the TBI group, suggesting that HBO treatment inhibits expression of CXCL1 and CXCR2 by downregulating the expression of p-ERK, p-JNK, and p-NF-κB.

Discussion

Numerous chemokines have been detected in both TBI animal models and patients, including CCL2, CCL3, CXCL1, CXCL2, CXCL10, CXCL12, chemokine (C–C motif) receptor (CCR) 2, CCR5, CXCR4, and chemokine (C-X3-C motif) receptor 1 (Morganti-Kossmann et al. 2019; Jassam et al. 2017; Chen et al. 2016; Dyhrfort et al. 2019; Gyoneva and Ransohoff. 2015; Perez-Polo et al. 2013; Thelin et al. 2017). The cytokine CXCL1 contributes to neuroinflammation primarily through binding to CXCR2 (Valles et al. 2006; Wu et al. 2015). In TBI model rats, local expression of CXCL1 peaked within 4 h after injury and was sustained for hours thereafter (Dalgard et al. 2012). Inflammatory profiling by multi-plex enzyme-linked immunosorbent assay revealed increased inflammatory markers, including CXCL1, in plasma and in the cerebellum post-injury alongside behavioral deficits up to 7 days following a mild, closed-head weight-drop injury (Fraunberger et al. 2020). In human TBI, high protein levels were observed for e.g., CXCL1, CXCL10 in the injured brain (Dyhrfort et al. 2019).

In this study, we found that the number of apoptotic cells increased in the cortical vicinity of the injured area after TBI, and neurological function was impaired. The expression of CXCL1 and CXCR2 increased and peaked at 1 and 3 days after TBI, and cell localization experiments revealed that CXCL1 was mainly expressed in astrocytes, while CXCR2 was mainly expressed in neurons. The decrease in nerve cell apoptosis and improvement in neurological with application of a CXCR2 antagonist suggested that CXCL1–CXCR2 mediated the interaction between activated astrocytes and neurons following TBI, aggravating secondary injury. Collectively, these results suggest that CXCL1 signaling may induce a sustained intracerebral inflammatory response in the hours and days following TBI. Our previous studies showed that during inflammation, CXCL1 expression in primary cultured astrocytes increased through the NF-κB, ERK, and JNK signaling pathways, and that CXCL1 binding to its receptor mediated the inflammatory response, thereby playing an important role in TBI possibly (Liu et al. 2018). We further found that the expression of p-ERK, p-JNK, and p-NF-κB increased after TBI, while ERK, JNK, and NF-κB inhibitors decreased the expression of CXCL1 and CXCR2,
We thank the staff members of our team for their treatment of TBI.

HBO treatment exerts neuroprotective effects after neurological injury, modulating multiple neuroinflammatory pathways may be one of the mechanisms. After spinal cord injury in rats, HBO treatment inhibits the high-mobility group protein B1/ NF-κB and Toll-like receptor 2 signaling pathways, thus reducing secondary injury caused by inflammation and promoting the recovery of neurological function (Tan et al. 2014; Yang et al. 2013). Some animal studies have shown that HBO treatment reduced the expression of caspase-3, TNF-α, IL-6, and IL-1β by inhibiting the Toll-like receptor 4/NF-κB signaling pathway after TBI in rats, thus reducing the secondary injury caused by inflammation after TBI and promoting the recovery of neurological function (Meng et al. 2016). IL-10 plays an important role in the neuroprotective effects of HBO on TBI, and IL-10 deficiency leads to increase brain damage after TBI and decrease suppression of neuroinflammation by HBO treatment (Chen et al. 2014). HBO therapy can reduce nerve cell apoptosis in the acute phase of TBI by regulating the Akt/GSK3β/β-catenin pathways (He et al. 2019). We showed in a previous study that HBO therapy modulates the NF-κB/MAPKs-CXCL1 signaling pathways to suppress inflammation induced by lipopolysaccharide in primary astrocytes of neonatal rats (Liu et al. 2018). In the present study, we found that HBO therapy could downregulate the expression of p-ERK, p-JNK, p-NF-κB, CXCL1 and CXCR2, while reducing nerve cell apoptosis and improving the neurological functions of TBI rats, ultimately alleviating secondary injury. In summary, the results of this study suggest that HBO therapy may play a neuroprotective role by regulating the NF-κB/MAPKs (JNK, and ERK)-CXCL1 inflammatory pathway.

Conclusion

Our study demonstrates that the NF-κB/MAPKs (JNK and ERK)-CXCL1 signaling pathways may mediate neuroinflammation following TBI that exacerbating secondary injury. HBO therapy can exert neuroprotective effects by targeting the NF-κB/MAPKs-CXCL1 inflammatory pathways after TBI. This study may provide theoretical and experimental evidence for the clinical use of HBO in the treatment of TBI.

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Author contributions AX, HH, WY, YL, HW, SL performed the experiments. SL conceived and designed the study. YL and HW analyzed the data. All authors read and approved the final manuscript.

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Availability of data and materials All data used during the current study available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Ethics approval consent to participate The protocol of this study was approved by the Experimental Animal Center of Nantong University (permission number: 20191106-003).

Consent for publication Not applicable.

Competing interests The authors declare that they have no conflicts of interest related to this study.

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