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INTERLEUKIN-10 MEDIATES THE NEUROPROTECTION OF HYPERBARIC OXYGEN THERAPY AGAINST TRAUMATIC BRAIN INJURY IN MICE

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Abstract—The aim of present study was to elucidate the role of Interleukin-10 (IL-10) in the neuroprotection of hyperbaric oxygen (HBO) against traumatic brain injury (TBI) in mice. The TBI in mice was induced by controlled cortical impact (CCI). HBO was given for 1 h at 2.0 absolute atmosphere (ATA) in 100% O_2. HBO enhanced the serumal and cerebral IL-10 protein levels in both sham-operated and TBI mice. HBO therapy after TBI reduced lesion volume, attenuated cerebral edema, improved neurological status including motor and cognitive function, inhibited apoptosis evidenced by decreased ratio of cleaved caspase-3 (C3) to pro-C3 and Bax expression and increased bcl-2 expression, and attenuated inflammation marked by reduced expression of IL-1β, IL-6, macrophage inflammatory protein-2 (MIP-2), and monocyte chemoattractant protein-1 (MCP-1) and activity of matrix metalloproteinase-9 (MMP9). In addition, HBO after TBI improved the blood–brain barrier, and upregulated the expression of tight junction proteins including zonula occludens-1 (ZO-1) and claudin-5. IL-10 deficiency aggravated TBI-induced damage in the brain and abrogated the beneficial effects of HBO on neuroinflammation, apoptosis, and edema after TBI. IL-10 deficiency itself had no significant effect on brain water content and neurological status. In conclusion, IL-10 played an important role in the neuroprotection of HBO therapy against TBI in mice. © **2014 Published by Elsevier Ltd. on behalf of IBRO.**

Key words: interleukin-10, hyperbaric oxygen, traumatic brain injury, apoptosis, inflammation, edema.

INTRODUCTION

Traumatic brain injury (TBI), as a serious public healthcare burden, is the major cause for trauma-related death and disability in industrialized countries affecting over 55 million people worldwide (Langlois et al., 2006). TBI causes cell death and neurologic dysfunction through both direct physical disruption of the brain (primary injury), as well as through delayed and potentially reversible molecular and cellular pathophysiological mechanisms, which cause progressive white and gray matter damage (secondary injury) (Bramlett and Dietrich, 2007). Hyperbaric oxygen (HBO) therapy, as a treatment by which 100% oxygen is administered to a patient at a pressure greater than atmospheric pressure at sea level, has been shown to provide obvious neuroprotection in brain-injured patients and animals (Palzur et al., 2008; Lin et al., 2008, 2012; Prakash et al., 2012; Sahni et al., 2012), but its efficacy and mechanisms of in TBI have not been well established.

Interleukin-10 (IL-10), a cytokine with anti-inflammatory properties, negatively modulates pro-inflammatory cascades at multiple levels. In the past few years, the beneficial effects of IL-10 have been demonstrated in several neuroinflammatory disease models, such as experimental autoimmune encephalomyelitis, traumatic or excitotoxic spinal cord injury, stroke and Parkinson's disease (Cua et al., 2001; Frenkel et al., 2005; Qian et al., 2006; Thompson et al., 2013). Treatment of rats subjected to lateral fluid percussion-induced TBI with IL-10 has been shown to improve neurological recovery and reduced levels of IL-1β and tumor necrosis factor α (TNFα) in brain tissues (Knoblach and Faden, 1998).

HBO therapy enhanced IL-10 levels in rats subjected to lateral fluid percussion-induced TBI (Lin et al., 2012). In this work, we found HBO therapy enhanced IL-10 levels in mice subjected to controlled cortical impact (CCI)-induced TBI. To elucidate the role IL-10 in the neuroprotection of HBO therapy against TBI, we tested the effect of HBO therapy in IL-10 knockout mice after CCI-induced TBI.

EXPERIMENTAL PROCEDURES

Animals

Wild-type mice (WT) and IL-$10^{-/-}$ mice (KO) were purchased from Charles River (Charles River Laboratories International, Shanghai, China). All animals were on a C57BL/6 background, bred, and housed

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Abbreviations: ATA, absolute atmosphere; BBB, blood–brain barrier; BWC, brain water content; C3, caspase-3; CCI, controlled cortical impact; D.I., discrimination index; EB, Evans Blue; ELISA, enzyme-linked immunosorbent assay; H&E, hematoxylin and eosin; HBO, hyperbaric oxygen; IL-10, Interleukin-10; KO, IL-10 knockout mice; NOR, novel object recognition; MCP-1, monocyte chemoattractant protein-1; MIP-2, macrophage inflammatory protein-2; MMP9, matrix metalloproteinase-9; MWM, Morris Water Maze; TBI, traumatic brain injury; WT, wild-type mice; ZO-1, zonula occludens-1.

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under specific pathogen-free conditions. All the mice were entrained to controlled temperature (23–25 °C), 12-h light/dark cycles (light, 08:00–20:00 h; darkness, 20:00–08:00 h), and free access to food and tap water. All the mice used in present study received humane care in compliance with institutional animal care guidelines, and were approved by the Local Institutional Committee. All experimental procedures were performed in accordance with institutional animal care guidelines. Before experiments, the female mice had vaginal smears performed to confirm similar cycle status.

Study design

Experiment 1. Female C57BL/6 mice (8 weeks old) were exposed to the CCI-induced TBI. 3 h after CCI surgery, mice were given 1 h of HBO at 2.0 absolute atmosphere (ATA) in 100% O_2 or room air. 2, 6 and 12 h later, mice were sacrificed; serum and the ipsilateral cortex were collected for the determination of IL-10 levels by enzyme-linked immunosorbent assay (ELISA).

Experiment 2. Female wild-type mice (WT) and IL-$10^{-/-}$ mice (KO) were exposed to the CCI-induced TBI. 3 h after TBI surgery, mice were given 1 h of HBO at 2.0 ATA in 100% O_2 or room air for 5 consecutive days. 4 weeks after TBI, lesion volume was evaluated after hematoxylin and eosin (H&E) staining.

Experiment 3. Female wild-type mice (WT, 8 weeks old) and IL-$10^{-/-}$ mice (KO, 8 weeks old) were exposed to the CCI-induced TBI. 3 h after TBI surgery, mice were given 1 h of HBO at 2.0 ATA in 100% O_2 or room air for 5 consecutive days. The ipsilateral cortex was collected for the determination of IL-1β, IL-6, macrophage inflammatory protein-2 (MIP-2), and monocyte chemoattractant protein-1 (MCP-1) levels by ELISA, matrix metalloproteinase-9 (MMP9) activity by gelatin zymography, cleaved caspase-3 (C3), pro-C3, bcl-2, Bax, zonula occludens-1 (ZO-1), and claudin-5 protein expression by Western blotting analysis. The blood–brain barrier (BBB) permeability was evaluated by measuring Evans Blue (EB) extravasation. Brain water content (BWC) of the ipsilateral cortex was quantified using the wet-dry method.

Experiment 4. Female wild-type mice (WT) and IL-$10^{-/-}$ mice (KO) were exposed to the CCI-induced TBI. 3 h after TBI surgery, mice were given 1 h of HBO at 2.0 ATA in 100% O_2 or room air for 5 consecutive days. Spatial learning and memory was assessed using an acquisition paradigm of the Morris Water Maze (MWM) test on post-injury days 14, 15, 16, and 17.

Experiment 5. Female wild-type mice (WT) and IL-$10^{-/-}$ mice (KO) were exposed to the CCI-induced TBI. 3 h after TBI surgery, mice were given 1 h of HBO at 2.0 ATA in 100% O_2 or room air for 5 consecutive days. Retention or intact memory was assessed by the novel object recognition (NOR) test on post-injury day 21.

Experiment 6. Female wild-type mice (WT) and IL-$10^{-/-}$ mice (KO) were exposed to the CCI-induced TBI. 3 h after TBI surgery, mice were given 1 h of HBO at 2.0 ATA in 100% O_2 or room air for 5 consecutive days. Motor performance was assessed at 1, 3, 7, and 14 days post-injury using the beam walk task. Motor performance was started 6 h after HBO therapy on post-injury days 1 and 3.

Experiment 7. Female wild-type mice (WT) and IL-$10^{-/-}$ mice (KO) were exposed to the CCI-induced TBI. 1 h after TBI surgery, mice were subcutaneously injected with IL-10 (100 μg/mouse) or 0.9% physiological saline for 5 consecutive days. Lesion volume was evaluated; brain BWC of the ipsilateral cortex was quantified; functional assessment was performed as above.

Model of TBI: CCI

Mice were anesthetized with isoflurane evaporated in a gas mixture containing 70% N_2O and 30% O_2 and administered through a nose mask (induction at 4% and maintenance at 2%). Monitoring respiration rate and pedal withdrawal reflexes assessed the depth of anesthesia. Utilizing aseptic techniques, a midline scalp incision was made, and the skin and fascia were reflected to expose the skull. A craniotomy was made in the right hemisphere encompassing bregma and lambda and between the sagittal suture and the coronal ridge with a Micro motor hand piece and drill (UGO Basile S.R.L., Comerio, VA, Italy). The resulting bone flap was removed, and the craniotomy enlarged further with cranial rongeurs. A cortical contusion was produced on the exposed cortex using a controlled impactor device Impact One Stereotaxic impactor for CCI (MyNeurolab, St. Louis, MO, USA). Briefly, the impacting shaft was extended, and the impact tip was centered and lowered over the craniotomy site until it touched the dura mater. Then, the rod was retracted and the impact tip was advanced farther to produce a brain injury of moderate severity for mice (tip diameter, 4 mm; cortical contusion depth, 3 mm; impact velocity, 1.5 m/s). The impact tip was cleaned with sterile alcohol (75%) and disinfected further with cidex after each impact. Temperature was maintained at 37 ± 0.5 °C with a heating pad during surgery and monitored with a rectal probe throughout the surgery. Immediately after impact, the skin incision was closed with nylon sutures, and 2% lidocaine jelly was applied to the lesion site to minimize any possible discomfort. Sham animals underwent the same procedure as injured mice except for the impact.

Materials

Chemicals, reagents, and drugs were purchased from Sigma Chemical (St. Louis, MO, USA) unless otherwise stated.

HBO therapy

Mice were placed in a custom-made pressure chamber of transparent acrylic plastic (701 Space Research Institute, Beijing, China) and given 1 h of HBO at 2.0 ATA in 100% O_2. The chamber was flushed with 100% oxygen at a rate of 5 L/min to avoid carbon dioxide accumulation. The pressure chamber temperature was maintained between 23 and 25 °C. To minimize the effects of diurnal variation, all HBO exposures were started at approximately 2:00 PM. The non-HBO mice were placed in the same chamber breathing room air.

Measurement of lesion volume

4 weeks after TBI, mice were anesthetized intraperitoneally with chloral hydrate, and perfused transcardially first with saline solution, followed by 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS, pH 7.4). The brains were removed and post-fixed in 4% paraformaldehyde at room temperature for 2–3 days. The brain tissue was cut into seven equally spaced (1 mm) coronal blocks, and processed for paraffin sectioning. A series of adjacent 6-μm-thick sections were cut from each block in the coronal plane and stained with H&E. Lesion volume was estimated based on the Cavalieri method of unbiased stereology using Stereologer 2000 software (Systems Planning and Analysis, Alexandria, VA, USA). The indirect lesion area was calculated (the intact area of the ipsilateral hemisphere was subtracted from the area of the contralateral hemisphere), and the lesion volume was presented as a volume percentage of the lesion compared with the contralateral hemisphere.

Assessment of cerebral edema

BWC, a sensitive measure of cerebral edema, was quantified using the wet-dry method. BWC was estimated in 3-mm coronal sections of the ipsilateral cortex, centered upon the impact site. Tissue was immediately weighed (wet weight), then dehydrated at 65 °C. The sample was reweighed 2 days later to obtain a dry weight. The percentage of tissue water content was calculated using the following formula: BWC = [(wet weight) − (dry weight)/wet weight] × 100%.

Functional assessment

MWM. Spatial learning and memory was assessed using an acquisition paradigm of the MWM test on post-injury days 14, 15, 16, and 17, as described previously (Fox et al., 1998; Loane et al., 2009). A white circular pool was divided into four quadrants using the computer-based AnyMaze video-tracking system (Stoelting Co., Wood Dale, IL, USA) and the platform was hidden in one quadrant (southwest) 14 inches from the side wall. Spatial learning and memory performance was assessed by determining the latency (in seconds) to locate the submerged hidden platform with a 90-s limit per trial. Four trials with an intertrial interval of 15 s were performed a day.

Reference spatial memory was assessed by a probe trial carried out on post-injury day 18 (24 h after the final learning trial), as the time spent (in seconds) with a 60-s limit in the quadrant where the platform had been hidden during the acquisition phase. A visual cue test was subsequently performed on post-injury day 18 using a flagged platform placed on the platform in one of the quadrants (with a 90-s limit per trial), and latency (in seconds) to locate the flagged platform was recorded.

NOR. Retention or intact memory was assessed by the NOR test on post-injury day 21. The apparatus consists of an open field (22.5 × 22.5 cm) with two adjacently-located imaginary circular zones, as previously designed (Bevins and Besheer, 2006). The cognitive outcomes were calculated as the "discrimination index" (D.I.) using the following formula: % D.I. = (time spent in novel object zone)/(time spent in old object zone + time spent in novel object zone).

Motor function. Motor performance was assessed on post-injury days 1, 3, 7, and 14 using the beam walk task, as previously described (Fox et al., 1998). Briefly, mice were trained to cross a narrow wooden beam 6-mm wide and 120 mm in length, which was suspended 300 mm above a 60-mm thick foam rubber pad. The number of foot-faults for the right hind-limb was recorded over 50 steps and a basal level of competence at this task was established before surgery with an acceptance level of < 10 faults per 50 steps.

All functional studies were performed by an investigator blinded to the groups.

ELISA

The brain tissue homogenates were obtained from the ipsilateral cortex. The concentrations of IL-1β, IL-10, IL-6, MIP-2, and MCP-1 were measured using specific ELISA kits according to the manufacturers' instructions (IL-10, IL-1β, IL-6, and MIP-2 from R&D Systems, Minneapolis, MN, USA; MCP-1 from Invitrogen, Camarillo, CA, USA). The final result was normalized to protein concentration.

Gelatin zymography

The brain tissue homogenates were obtained from the ipsilateral cortex. Equal amounts of protein were separated on a 10% Tris–glycine gel copolymerized with 0.1% gelatin as substrate. After separation, the gel was washed twice in distilled water and then, proteins within the gel were renatured by incubation with 2.5% Triton-X-100 buffer for 1 h at room temperature. After incubating with developing buffer (0.05 mol/L Tris–HCl pH 7.5, 0.2 mol/L NaCl, 5 mmol/L $CaCl_2$, 0.05% Brij-35, and 0.2 mmol/L NaN_3) at 37 °C for 24 h, the gel was stained with 0.05% Coomassie R-250 dye for 30 min followed by destaining. Gelatinolytic activity was indicated by the detection of clear bands at the appropriate molecular weight.

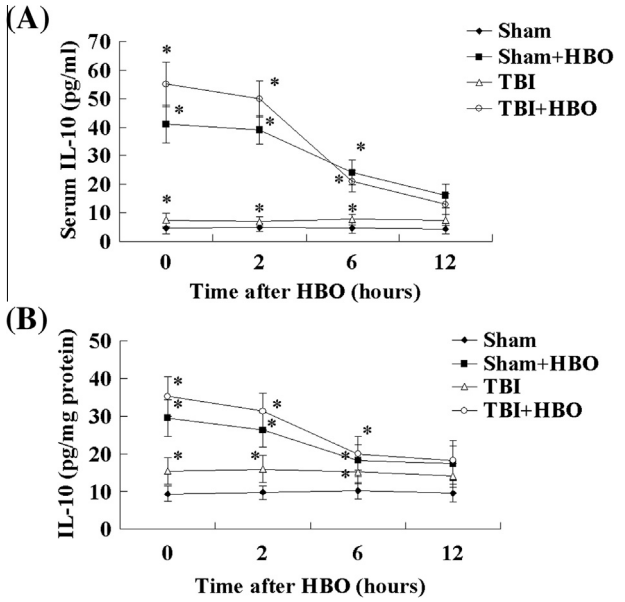

Fig. 1. The effects of HBO therapy on IL-10 protein levels in mice with TBI. IL-10 protein levels in serum (A) and ipsilateral cortices (B) were analyzed by the ELISA method. $n = 9$ in each group; TBI, traumatic brain injury; IL-10, Interleukin-10; HBO, hyperbaric oxygen; Values are means ± SD. $^{*}P < 0.05$ versus sham group.

Western blotting analysis

The protein concentration was determined with bovine serum albumin as a standard by a Bradford assay. Equal amount of protein preparations (15 μg in 10 μl buffer) were run on sodium dodecyl sulfate (SDS)–polyacrylamide gels, electrotransferred to polyvinylidine difluoride membranes, and blotted with primary antibodies against cleaved C3, pro-C3, bcl-2, Bax, ZO-1, and claudin-5 (Santa Cruz Biotechnology, Inc., Paso Robles, CA, USA) overnight at 4 °C using slow rocking. Then, they were blotted with HRP-conjugated secondary antibody (1:10,000) and HRP-conjugated monoclonal antibody against β-actin (1:8000). Immunoreactive bands were detected by a chemiluminescent reaction (ECL kit, Amersham Pharmacia, Piscataway, NJ, USA).

The protein levels of bcl-2, Bax, ZO-1, and claudin-5 were adjusted as relative values to β-actin. The ratio of cleaved C3 expression to pro-C3 expression was calculated to assess apoptosis.

Evaluation of BBB permeability

BBB permeability was evaluated by measuring EB extravasation. Briefly, EB dye (4 ml/kg in 2% saline) was administered via the tail vein and allowed to circulate for

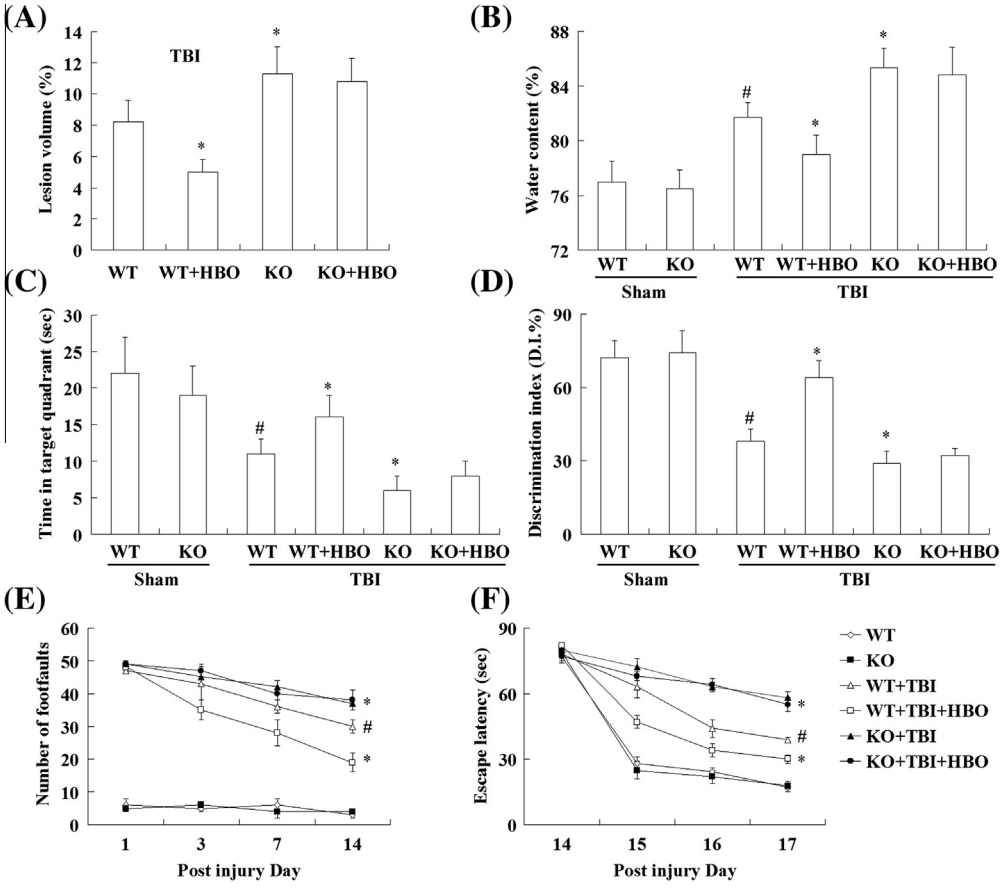

Fig. 2. The effects of HBO therapy on functional recovery in wild-type and IL-10 knockout mice with TBI. Lesion volume (A) was evaluated after H&E staining. Brain water content (B) of the ipsilateral cortex was quantified using the wet-dry method. Reference memory (C) was assessed using the MWM probe test. Retention memory (D) was assessed using novel object recognition (NOR) test. Motor co-ordination deficits (E) were assessed using a beam walk test. Spatial learning and working memory (F) was assessed using a MWM test. $n = 7$ in each group; TBI, traumatic brain injury; IL-10, Interleukin-10; HBO, hyperbaric oxygen; WT, wild-type mice; KO, IL-10 knockout mice; MWM, Morris Water Maze; Values are means ± SD. $^{*}P < 0.05$ versus WT with TBI; $^{\#}P < 0.05$ versus WT with sham.

60 min. To wash out intravascular EB, the animals were then perfused with saline through the left ventricle at a pressure of 110 mmHg until colorless fluid was obtained from the right atrium. Brains were removed, and ipsilateral hemispheres were cut into 4-mm-thick sections (2 mm from the frontal pole) and weighed. For the extraction of EB from brain tissues, hemispheres were placed in 1 ml of 60% (w/v) trichloroacetic acid and homogenized by sonication. Homogenates were centrifuged for 15 min, and the supernatants were diluted with ethanol (1:4). The absorbance of each supernatant for the EB dye was recorded at 620 nm using a spectrophotometer. EB concentrations were calculated as μg/g brain tissue against a standard curve.

Statistical analysis

All data are expressed as mean ± SD. Comparison among groups was analyzed using a two-way analysis of variance followed by the Bonferroni *t*-test. $P < 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS 11.0.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

The effect of HBO therapy on IL-10 protein levels in mice with TBI

IL-10 protein levels in both serum (Fig. 1A) and ipsilateral cortices (Fig. 1B) were higher in TBI mice than that in sham-operated mice. HBO therapy resulted in upregulation of IL-10 protein levels of serum and ipsilateral cortices in both TBI mice and sham-operated mice. The effect of HBO therapy on IL-10 protein levels lasted for 6 h at least in mice.

The effect of HBO therapy on functional recovery in wild-type and IL-10 knockout mice with TBI

HBO in wild-type mice with TBI reduced lesion volume (Fig. 2A), decreased water content of ipsilateral cortices (an indicator of brain edema, Fig. 2B), and improved motor and cognitive function marked by restored time in target quadrant (Fig. 2C), D.I. (Fig. 2D), number of foot-faults (Fig. 2E), and escape latency (Fig. 2F).

Compared to wild-type mice, TBI in IL-10 knockout mice induced larger lesion volume, more water content

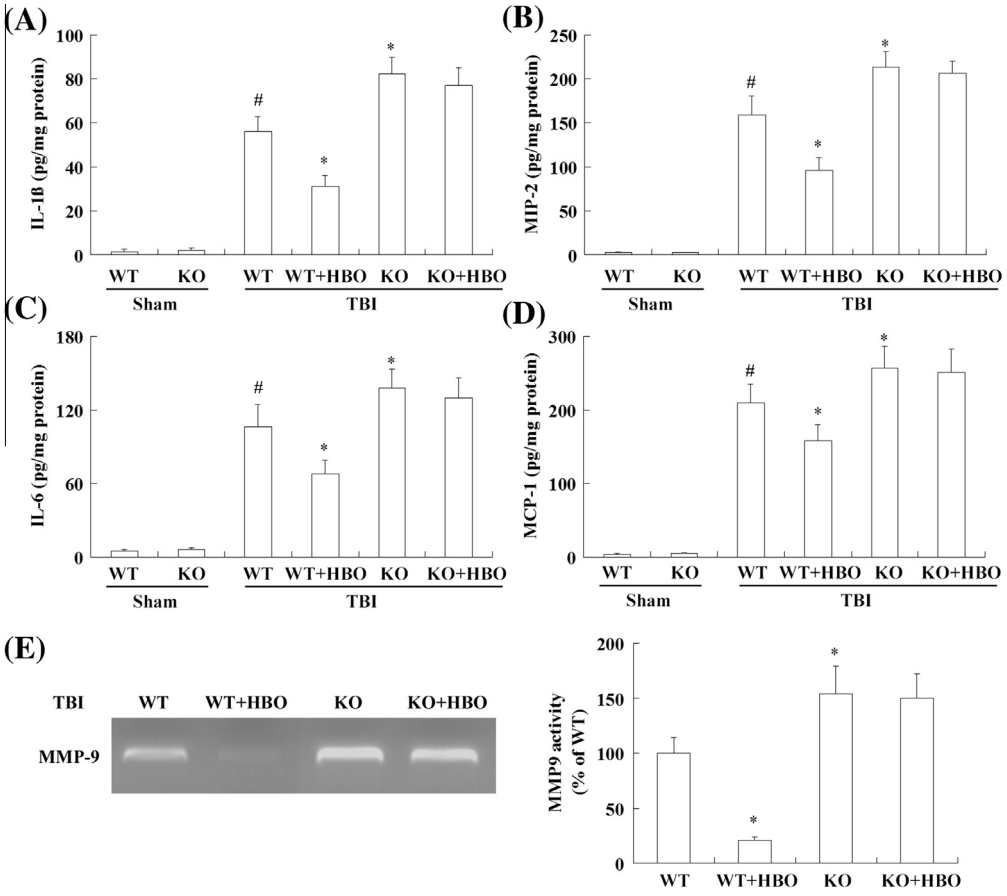

Fig. 3. The effects of HBO therapy on levels of inflammatory cytokines in wild-type and IL-10 knockout mice with TBI. IL-1β (A), MIP-2 (B), IL-6 (C), and MCP-1 (D) protein levels were determined by the ELISA method. MMP9 activity (E) was determined by gelatin zymography. $n = 7$ in each group; TBI, traumatic brain injury; IL-10, Interleukin-10; HBO, hyperbaric oxygen; WT, wild-type mice; KO, IL-10 knockout mice; MIP-2, macrophage inflammatory protein-2; MMP9, matrix metalloproteinase-9; MCP-1, monocyte chemoattractant protein-1; Values are means ± SD. $^{*}P < 0.05$ versus WT with TBI; $^{\#}P < 0.05$ versus WT with sham.

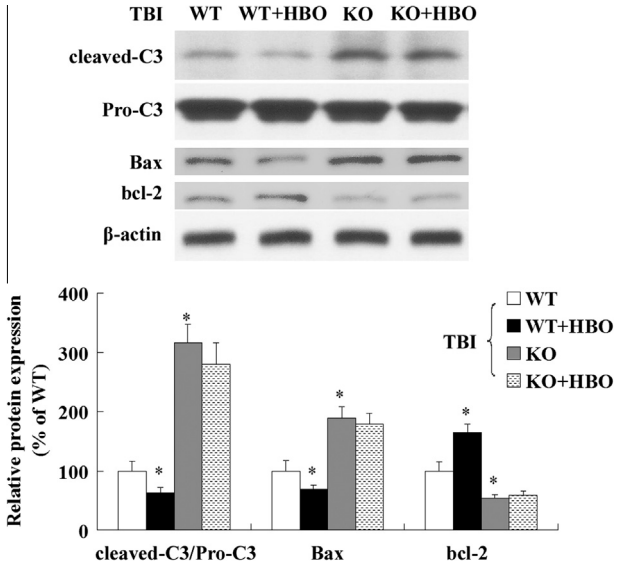

Fig. 4. The effects of HBO therapy on apoptosis in wild-type and IL-10 knockout mice with TBI. Protein expression of cleaved-C3, pro-C3, Bax, and bcl-2 were determined by Western blotting analysis. A Western blot representative of three independent experiments was shown. TBI, traumatic brain injury; IL-10, Interleukin-10; HBO, hyperbaric oxygen; WT, wild-type mice; KO, IL-10 knockout mice; C3, caspase-3; Values are means ± SD. $^{*}P < 0.05$ versus WT with TBI.

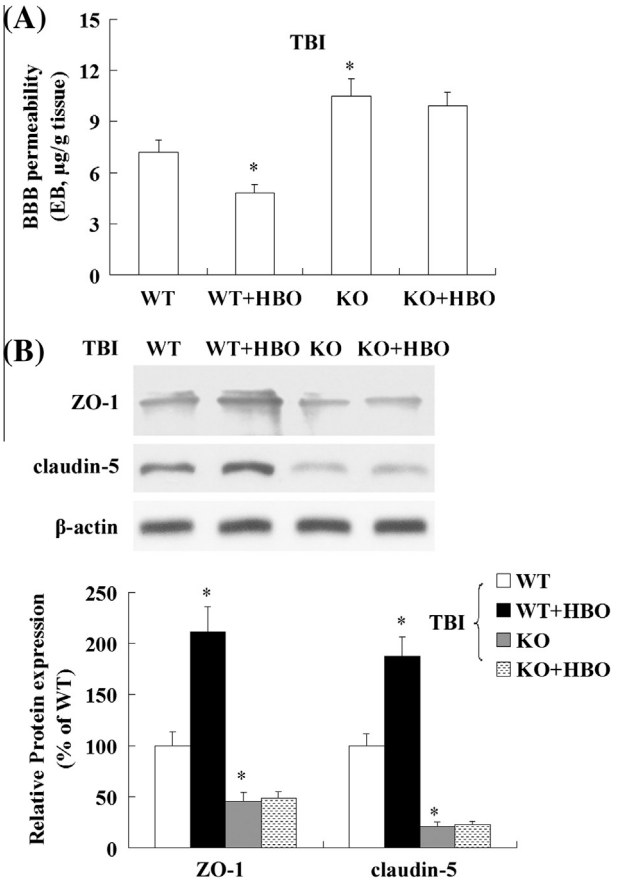

Fig. 5. The effects of HBO therapy on BBB permeability in wild-type and IL-10 knockout mice with TBI. BBB permeability (A) was evaluated by measuring EB extravasation. $n = 7$ in each group; Protein expression (B) of ZO-1 and claudin-5 were determined by Western blotting analysis. A Western blot representative of three independent experiments was shown. TBI, traumatic brain injury; IL-10, Interleukin-10; HBO, hyperbaric oxygen; WT, wild-type mice; KO, IL-10 knockout mice; BBB, blood–brain barrier; ZO-1, zonula occludens-1; Values are means ± SD. $^{*}P < 0.05$ versus WT with TBI.

of ipsilateral cortices, and aggravated motor and cognitive dysfunction marked by less time in target quadrant, lower D.I., more number of foot-faults, and increased latency to find the submerged platform.

HBO in IL-10 knockout mice with TBI had no significant effect on lesion volume, water content of ipsilateral cortices, or motor and cognitive dysfunction.

Water content of ipsilateral cortices and motor and cognitive function was similar between wild-type mice and IL-10 knockout mice.

The effect of HBO therapy on levels of inflammatory cytokines in wild-type and IL-10 knockout mice with TBI

HBO in wild-type mice with TBI suppressed inflammation marked by reduced protein levels of IL-1β (Fig. 3A), MIP-2 (Fig. 3B), IL-6 (Fig. 3C), and MCP-1 (Fig. 3D) and reduced activity of MMP9 (Fig. 3E) in ipsilateral cortices.

Compared to wild-type mice, IL-10 knockout aggravated TBI-induced reduction of protein levels of IL-1β, MIP-2, IL-6, and MCP-1 and activity of MMP9 in ipsilateral cortices.

HBO in IL-10 knockout mice with TBI had no significant effect on protein levels of IL-1β, MIP-2, IL-6, and MCP-1 or activity of MMP9 in ipsilateral cortices.

Protein levels of IL-1β, MIP-2, IL-6, and MCP-1 in ipsilateral cortices were similar between wild-type mice and IL-10 knockout mice.

The effect of HBO therapy on apoptosis in wild-type and IL-10 knockout mice with TBI

HBO in wild-type mice with TBI inhibited apoptosis (Fig. 4) marked by reduced ratio of cleaved-C3 to pro-C3, reduced the expression of Bax, and increased the expression of bcl-2 ($P < 0.05$) in ipsilateral cortices.

Compared to wild-type mice, IL-10 knockout aggravated TBI-induced apoptosis marked by higher ratio of cleaved-C3 to pro-C3, higher expression of Bax, and lower expression of bcl-2 in ipsilateral cortices.

HBO in IL-10 knockout mice with TBI had no significant effect on apoptosis in ipsilateral cortices.

The effect of HBO therapy on BBB permeability in wild-type and IL-10 knockout mice with TBI

BBB permeability was determined by EB dye extravasation. HBO in wild-type mice with TBI led to marked reduction in EB content in the ipsilateral hemisphere (Fig. 5A) and enhancement in ZO-1

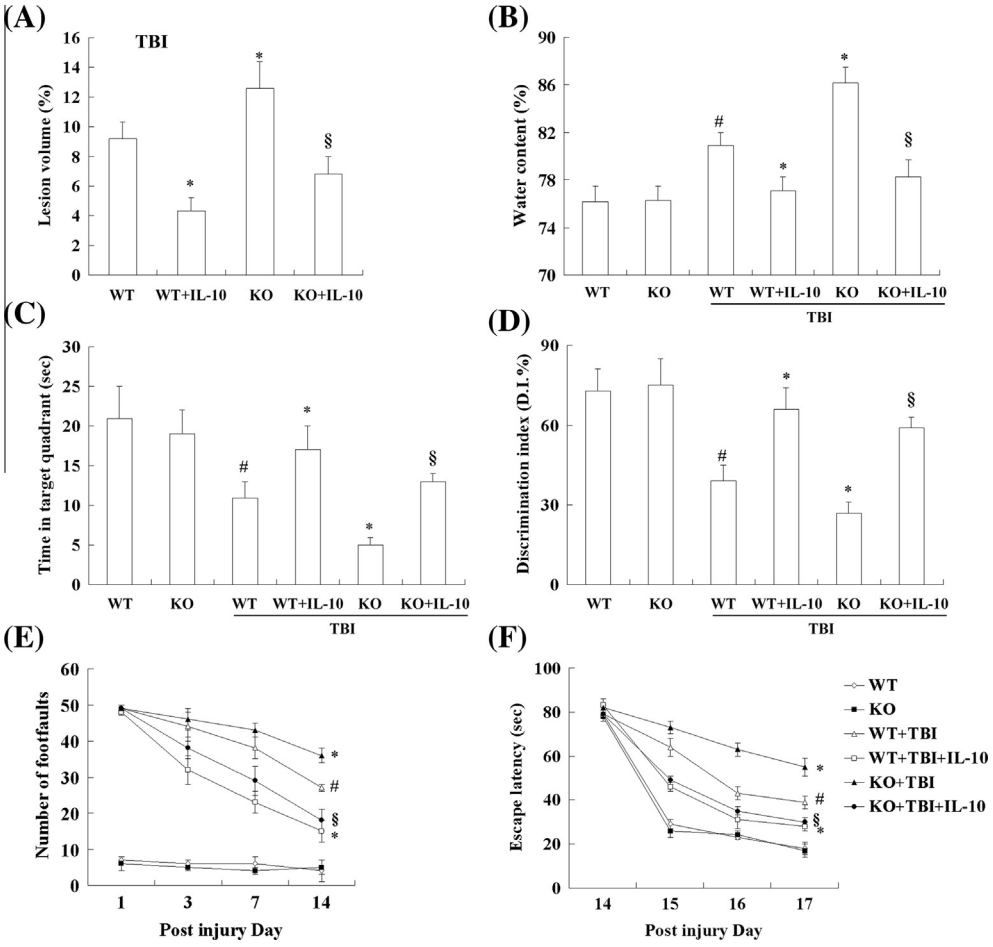

Fig. 6. The effects of treatment with IL-10 on functional recovery in wild-type and IL-10 knockout mice with TBI. Lesion volume (A) was evaluated after H&E staining. Brain water content (B) of ipsilateral cortex was quantified using the wet-dry method. Reference memory (C) was assessed using the MWM probe test. Retention memory (D) was assessed using novel object recognition (NOR) test. Motor co-ordination deficits (E) were assessed using a beam walk test. Spatial learning and working memory (F) was assessed using a MWM test. $n = 7$ in each group; TBI, traumatic brain injury; IL-10, Interleukin-10; WT, wild-type mice; KO, IL-10 knockout mice; MWM, Morris Water Maze; Values are means ± SD. $^{*}P < 0.05$ versus WT with TBI; $^{\#}P < 0.05$ versus WT with sham; $^{\S}P < 0.05$ versus KO with TBI.

(Fig. 5B) and claudin-5 (Fig. 5B) protein expression in ipsilateral cortices.

Compared to wild-type mice, IL-10 knockout aggravated TBI-induced BBB dysfunction marked by higher EB content in the ipsilateral hemisphere and lower protein expression of ZO-1 and claudin-5 in ipsilateral cortices.

HBO in IL-10 knockout mice with TBI had no significant effect on BBB permeability or protein expression of ZO-1 and claudin-5.

The effect of treatment with IL-10 on functional recovery in wild-type and IL-10 knockout mice with TBI

Treatment with IL-10 in both wild-type mice and IL-10 knockout mice with TBI reduced lesion volume (Fig. 6A), decreased water content of ipsilateral cortices (an indicator of brain edema, Fig. 6B), and improved motor and cognitive function marked by restored time in target quadrant (Fig. 6C), D.I. (Fig. 6D), number of foot-faults (Fig. 6E), and escape latency (Fig. 6F).

DISCUSSION

Our present study showed that HBO therapy provided neuroprotection and promoted functional recovery against TBI by attenuating inflammation, inhibiting apoptosis, and reducing cerebral edema. HBO enhanced IL-10 production in brain tissue. IL-10 deficiency aggravated TBI-induced motor and cognitive dysfunction, inflammation, apoptosis, and cerebral edema and abrogated the therapeutic effects of HBO in mice with TBI.

It was shown that IL-10 was elevated in the cerebrospinal fluid and serum of patients with TBI (Csuka et al., 1999). In this work, it was found that IL-10 levels in serum and ipsilateral cortices were slightly but significantly elevated 4 h after CCI surgery; and HBO therapy obviously enhanced IL-10 production independently from CCI surgery. Up to now, there were little data about the mechanism of the modulation of HBO on IL-10 production, which required further investigation.

One of the integral features of TBI is the inflammatory reaction initiated and regulated by an array of pro- and

anti-inflammatory cytokines (Woodcock and Morganti-Kossmann, 2013). Neuroinflammation is well established as a key secondary injury mechanism after TBI, and it has been long considered to contribute to the damage sustained following brain injury (Kumar and Loane, 2012; Acosta et al., 2013). Previous reports (Vlodavsky et al., 2006; Lin et al., 2012) and our result revealed that HBO therapy suppressed neuroinflammation after TBI evidenced by reduced protein levels of IL-1β, IL-6, MIP-2, and MCP-1. IL-10 deficiency abrogated the anti-inflammatory effect of HBO after TBI, which indicated that HBO suppressed neuroinflammation after TBI possibly via an IL-10-dependent mechanism.

Delayed neuronal cell death is a key element of the secondary injury, reflecting various types of neuronal programed cell death and contributes significantly to posttraumatic neurological deficits in experimental TBI (Yakovlev et al., 2001; Stoica and Faden, 2010). Caspase-mediated apoptosis was initially proposed as an important programed cell death pathway involved, based upon the demonstration of caspase activation in TBI models and neuroprotection shown with selective caspase inhibitors (Yakovlev et al., 1997; Knoblach et al., 2002). It was reported that HBO therapy alleviated neuronal apoptosis after TBI by reducing the formation of Bax and up-regulating the expression of Bcl-2 (Liu et al., 2006). Our results further demonstrated that HBO inhibited cleaving of C3 induced by TBI. The anti-apoptotic effects of IL-10 were also reported to be via downregulation of Bax and caspases-3 and upregulation of Bcl-2 (Londoño et al., 2011), which was also demonstrated in our work. IL-10 deficiency abrogated the anti-apoptotic effects of HBO after TBI, which indicated that HBO inhibited apoptosis after TBI via an IL-10-dependent mechanism.

Disruption of the BBB resulted in cerebral edema formation, which contributed to increased patient mortality and long-term disability after TBI (Unterberg et al., 2004; Donkin and Vink, 2010). Tight junction proteins, such as ZO-1 and claudin-5, are a hallmark for the integrity of the BBB that essentially contributes to its structural inviolacy. The alteration of tight junction proteins assembly may contribute to the loss of BBB integrity and BBB breakdown (Luh et al., 2010). In addition, accumulating evidence showed that inflammation and activation of MMPs might play key roles in the disruption of the BBB and brain edema formation after injury (Wang et al., 2000; Vajtr et al., 2009). HBO reduces BBB damage and edema after transient focal cerebral ischemia (Veltkamp et al., 2005) and attenuated cerebral edema in patients and animals with TBI (Niklas et al., 2004; Pérez-Espejo et al., 2009). In this work, HBO after TBI restored the expression of tight junction proteins, attenuated inflammation, and suppressed activity of MMP9. Pretreatment of monolayers with IL-10 reversed IFN-gamma-induced endothelial barrier dysfunction (Oshima et al., 2001). In this work, IL-10 deficiency aggravated TBI-induced BBB dysfunction and edema and abrogated the protection of HBO on BBB and anti-edema effect of HBO after TBI, which indicated that HBO restored BBB permeability and attenuated cerebral edema after TBI via an IL-10-dependent mechanism.

CONCLUSION

IL-10 mediates the neuroprotection of HBO therapy against TBI in mice through modulation of inflammation, apoptosis, and cerebral edema.

CONFLICTS OF INTEREST DISCLOSURE

The authors declare that we have no conflict of interest.

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