EJP
European Journal of Pain

ORIGINAL ARTICLE

Hyperbaric oxygen therapy attenuates neuropathic hyperalgesia in rats and idiopathic trigeminal neuralgia in patients

N. Gu[1]*, J.-Y. Niu[1]*, W.-T. Liu[2], Y.-Y. Sun[1], S. Liu[1,2], Y. Lv[1], H.-L. Dong[1], X.-J. Song[1,2], L.-Z. Xiong[1]

1 Department of Anesthesiology, Xijing Hospital, The Fourth Military Medical University, Xi'an, China
2 Department of Neurobiology, Parker University Research Institute, Dallas, USA

Correspondence
Li-Ze Xiong
Department of Anesthesiology, Xijing Hospital, 17 Changle W Road, Xi'an, Shaanxi 710032, China.
E-mail: lxiong@fmmu.edu.cn
Xue-Jun Song
E-mail: song@parker.edu

* These authors contributed equally to this work.

Funding sources
This work was supported by grants from National Natural Science Foundation of China (No.30628027 to X.-J. S. and no.30725039 to L.-Z.X.).

Conflicts of interest
There is no financial conflict of interest in publishing this article.

Accepted for publication
17 January 2012

doi:10.1002/j.1532-2149.2012.00113.x

Abstract

Background: Neuropathic pain after nerve injury is severe and intractable, and current drug and non-drug therapies offer very limited pain relief. Hyperbaric oxygen (HBO_2) has been clinically used for protection of the nervous system after acute injury. We investigated whether HBO_2 treatment could prevent and/or attenuate neuropathic pain in animals and in patients.
Methods: Mechanical allodynia and thermal hyperalgesia and neurochemical alterations of neuropathic pain were analysed in male, adult, Sprague-Dawley rats with sciatic nerve injury. Clinical trials were conducted in patients with idiopathic trigeminal neuralgia.
Results: Repetitive HBO_2 treatment [a combination of pressure at 3 atmosphere absolute (ATA) and pure oxygen] greatly inhibited behavioural signs of neuropathic pain manifested as thermal hyperalgesia and mechanical allodynia. Such an HBO_2 treatment also inhibited nerve injury-induced induction of c-Fos and activation of astrocytes and increased phosphorylation of NR2B receptor and the subsequent Ca^{2+}-dependent signals in rats. Neither high pressure (up to 3 ATA) nor pure oxygen alone resulted in analgesic effect. In clinical trials, one course of HBO_2 therapy (10 consecutive days) produced a rapid-onset, dose-dependent and long-lasting analgesic effects evidenced by the decreased doses of carbamazepine required for keeping patient pain at a minimum and decreased scores of visual analogue scales, which was used for patient's self-evaluation.
Conclusions: These findings support that HBO_2 therapy is an effective approach for treating neuropathic pain in both animals and human beings and suggest that neural protection, anti-inflammation and inhibition of nerve injury-induced altered neural activity may contribute to the analgesic effect of HBO_2 therapy.

1. Introduction

Hyperbaric oxygen (HBO_2) therapy has been clinically used for protection of the nervous system after acute injury. HBO_2 treatment may attenuate injury to nerve tissues after stroke (Zhang et al., 2005) and hippocampus ischaemia (Wada et al., 1996) as well as improving peripheral nerve repair and regeneration (Tibbles and Edelsberg, 1996; Sanchez, 2007). We have demonstrated that HBO_2 preconditioning can induce tolerance against spinal cord ischaemia (Dong et al., 2002; Nie et al., 2006). This is further supported by a recent study in forebrain ischaemia model (Yamashita et al., 2009). HBO_2 treatment may protect the injured nerve tissues through the following pathways: reducing tissue oedema and hypoxia, improving microcirculation and enhancing regeneration of micro-capillaries and the nerve tissues; inhibiting inflammatory responses (Wilson et al., 2006) and inflammatory factors such as interleukin-1, tumour necrosis factor and

What's already known about this topic?
- Hyperbaric oxygen (HBO_2) has been clinically used for protection of the nervous system after acute injury.

What does this study add?
- HBO_2 therapy is an effective approach for treating neuropathic pain in both animals and human beings
- Suggestion that neural protection, anti-inflammation and inhibition of nerve injury-induced altered neural activity may contribute to the analgesic effect of HBO_2 therapy.

cyclooxygenase-2 (Tibbles and Edelsberg, 1996; Waisman et al., 2003; Sanchez, 2007); and inducing adaptive protection against oxidative stress (Rothfuss and Speit, 2002). Recently, there are reports that HBO_2 therapy appears to be effective in some chronic pain conditions in patients such as headache (Di Sabato et al., 1993) and complex regional pain syndrome (Kiralp et al., 2004). In experimental animals, HBO_2 treatment can attenuate inflammatory pain (Sumen et al., 2001; Wilson et al., 2007; Zelinski et al., 2009). These reports suggest a potential role of HBO_2 in antinociception.

Neuropathic pain after nerve injury is severe and intractable and continues to pose a major challenge clinically. Neuropathic pain is initially triggered by nerve damage and manifests as pain-like behaviours and neurochemical alterations, hyperexcitability and enhanced synaptic plasticity in the dorsal root ganglion (DRG) and spinal dorsal horn (DH) neurons. The damaged nerves and their neighbouring neurons and non-neural cells are observed to have inflammation and de-myelination in DRG and a profound selective loss of GABAergic inhibition in the DH neurons (Bennett and Xie, 1988; Song et al., 1999, 2003, 2006, 2008; Moore et al., 2002; Ikeda et al., 2003; Kohno et al., 2003; Zheng et al., 2007; Woolf, 2010). Through decades of investigation, great progress has been made in understanding mechanisms of neuropathic pain, although they still remain elusive. However, current drugs, including opioid, and various non-drug therapies offer very limited pain relief clinically.

We hypothesized that as a therapy strategy, HBO_2, which can result in neural protection, might be effective in treating neuropathic pain through protecting the injured nerves from further damage and reversing or interrupting the injury-induced processing of nociception. We have recently extended our studies of HBO_2 therapy in neural protection to treatment of neuropathic pain in experimental animals with sciatic nerve injury and in patients with idiopathic trigeminal neuralgia (TN), a severe form of neuropathic facial pain presenting with paroxysmal, unilateral pain in one or more branches of the fifth cranial nerve (Krafft, 2008). We have found that HBO_2 therapy can greatly suppress idiopathic TN in patients as well as the nerve injury-induced neuropathic pain and the associated neurochemical alterations in rats. This study suggests that HBO_2 therapy may be an effective approach for treating neuropathic pain in animals and human being.

2. Materials and methods

2.1 Animal studies

2.1.1 Animals and neuropathic pain model

All experimental procedures were conducted in accordance with the recommendations of the International Association for the Study of Pain and the National Institute of Health Guide for the Care and Use of Laboratory Animals. The procedures were reviewed and approved by the Institutional Animal Care and Use Committee. Adult, male Sprague-Dawley rats (200–220 g weight at start of the experiment, purchased from Charles River Laboratories, Wilmington, MA, USA) were used in this study. All surgeries were performed under anaesthesia induced by sodium pentobarbital (40 mg/kg, i.p.). After surgery, the skin layers and muscle were sutured. Peripheral nerve injury was produced using the model of chronic constriction injury of the sciatic nerve (CCI). In brief, the left common sciatic nerve of each rat was exposed at the mid-thigh level. Proximal to the sciatic nerve's trifurcation, approximately 7 mm of nerve was separated of adhering tissue and four ligatures (4-0 chronic gut) were tied loosely around it with about 1 mm between ligatures. Animals in the sham group received surgery identical to those described but without nerve injury.

2.1.2 Assessment of thermal hyperalgesia and mechanical allodynia

Thermal hyperalgesia was assessed by measuring foot withdrawal latency to heat stimulation using a protocol as that we have described (Wang et al., 2005; Song et al., 2006, 2008). An analgesia meter (IITC Model 336 Analgesia Meter, Life Science, Series 8, Woodland Hills, CA, USA) was used to provide a heat source. In

brief, each rat was placed in a box containing a smooth, temperature-controlled glass floor. The heat source was focused on a portion of the hind paw, which was flush against the glass, and a radiant thermal stimulus was delivered to that site. The stimulus shut off when the hind paw moved (or after 20 s to prevent tissue damage). The intensity of the heat stimulus was maintained constant throughout all experiments. The elicited paw movement occurred at latency between 9 and 14 s in control animals. Thermal stimuli were delivered four times to each hind paw at 5- to 6-min intervals.

Mechanical allodynia was determined by measuring incidence of foot withdrawal in response to mechanical indentation of the plantar surface of each hind paw with a sharp, cylindrical probe with a uniform tip diameter of approximately 0.2 mm provided by an Electro von Frey (ALMEMO 2390-5, Anesthesiometer IITC Inc, Woodland Hills, CA, USA), using a protocol similar to that described previously (Song et al., 1999, 2003; Wang et al., 2005). In brief, the rat was placed on a metal mesh floor and covered with a transparent plastic dome ($20 \times 25 \times 15$ cm). The animal rested quietly in this situation after an initial few minutes of exploration. After about 15 min, the test was begun. Each filament was applied from underneath the metal mesh floor to the plantar surface of the foot. Electro von Frey was applied to 10 different spots spaced across nearby the entire extent of the paw (see Fig. 2A in Song et al., 1999). The duration of each stimulus was 3 s, in the absence of withdrawal, and the interstimulus interval was 10–15 s. The Electro von Frey was given in order of ascending force, with a given filament delivered to each spot alternatively from one paw to the other in sequence from the first to the 10th spot. The incidence of foot withdrawal was expressed as a percentage of the 10 applications of each stimulus as a function of force. The minimal force (in grams) that induced paw withdrawal was read off the display. Threshold of mechanical withdrawal in each rat was calculated by averaging the 10 readings and the force was converted into millinewtons (mN).

2.1.3 HBO_2, hyperbaric air (HBA) and pure oxygen treatment

The rats received HBO_2, HBA and pure oxygen (100% O_2) treatment, respectively. The protocol for HBO_2 was similar to that we have previously described (Xiong et al., 2000; Nie et al., 2006; Li et al., 2011). In brief, the animals received daily HBO_2 for seven consecutive days in an animal hyperbaric monochamber (701 Institute, DWC450-1150, Shanghai, China). The parameters of HBO_2 used were atmosphere absolute (ATA) at 1.5, 2.0 or 3.0, pure oxygen, each for 70 min. The compression and decompression were performed within 15 min (at a rate of 0.2 ATA/min). Before pressurization, the chamber was flushed with pure oxygen for 10 min to displace the ambient air. The oxygen concentration in the chamber was monitored using a calibrated oximeter and was maintained at ≥98%. Carbon dioxide was absorbed using calcium carbonate crystals. All of the exposures were started at the same time in order to minimize the possible impact of biologic rhythms. The chamber temperature was held at 23–26°C. The rats received daily treatment under normobaric room in 21% O_2 at 1 ATA for 70 min for seven consecutive days in the same chamber were served as the controls. The parameters of HBA treatment used were 3.0 ATA in 21% O_2 for 70 min. The parameters of pure oxygen treatment used were 1 ATA in 100% O_2 for 70 min. The protocols for HBA and pure oxygen treatment were the same as that for HBO_2.

2.1.4 Protein determinations

To quantify temporal changes of the phosphorylated protein levels of N-methyl-D-aspartate (NMDA) receptor subtypes NR1, NR2B, extracellular signal-regulated kinases (ERK), calmodulin-dependent kinase II (CaMKII), cyclic adenosine monophosphate (cAMP) response element-building (CREB) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in rat spinal cord, whole-cell protein extract lysates were used. The protocol was similar to that described previously (Song et al., 2008; Liu et al., 2010). Under anaesthesia and immediately after perfusion with phosphate buffered saline (PBS), the spinal cord tissue at segments L_1–L_6 was rapidly removed and homogenized in ice-cold (4°C) lysis buffer [50 mM Tris, pH 7.5, containing 150 mM NaCl, 1% Triton X-100, 0.5% deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 0.2 mM ethylenediaminetetraacetic acid (EDTA), 10 mM NaF, 10 μg/mL aprotinin, 1 μg/mL leupeptin, 10 μg/mL pepstatin, 0.4 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride and 1 mM sodium orthovanadate]. The protein concentration of the lysates was estimated using the method of BCA (with reagents from Pierce), and the total protein content between samples was equalized. Proteins were dissociated by heating at 100°C for 5 min in sample buffer (2% SDS, 100 mM dithiothreitol (DTT), 10% glycerol and 0.02% bromophenol blue) before loading on 10% or 12% SDS polyacrylamide gels to resolve protein bands. After transfer to nitrocellulose filters, the filters were blocked with 5% bovine serum albumin and then incubated overnight at 4°C

with the primary antibodies [phosphorylation of NR1 (pNR1) (Ser897), NR2B (pNR2B) (Tyr1472), 1:800 from Millipore Bioscience Research Reagents (Temecula, CA, USA); phosphorylation of ERK (pERK) (Thr202/yr204), 1:500; CaMKII (pCaMKII) (Thr286), 1:1000 from Cell Signaling Technology (Danvers, MA, USA); GAPDH, 1:1000 from Sigma (St Louis, MO, USA); glial fibrillary acidic protein (GFAP) and pCREB (Ser133) from Santa Cruz Biotechnology (SCT, Santa Cruz, CA, USA). The filters were developed using ECL reagents (PerkinElmer, Waltham, MA, USA) with secondary antibodies from R&D systems, Inc (Minneapolis, MN, USA). Data were analysed with a molecular imager (Gel Doc TM XR, 170–8170) and the associated software Quantity One-4.6.5 (Bio-Rad Laboratories, Hercules, CA, USA).

2.1.5 Immunohistochemistry

Under deep anaesthesia, the mice were transcardially perfused with PBS followed by 4% paraformaldehyde with 1.5% picric acid in 0.16 M PB (pH 7.2–7.4, 4°C), and then the L4 and/or L5 lumbar segment was dissected out and post-fixed in the same fixative overnight. The embedded blocks were sectioned (30 μm thick) and processed for immunofluorescence. Sections from each group (five rats in each group) were incubated with polyclonal antibodies of rabbit anti-c-Fos (1:100, sc-52, SCT) and anti-GFAP (1:500, ab7260, Abcam, Cambridge, UK), respectively. Rabbit IgG (1:200, Vector Laboratories, Burlinghame, CA, USA) was used as an isotype control. Morphologic details examined with a confocal microscope (FluoView FV1000, Olympus Co., Tokyo, Japan). Number of Fos-like immunoreactive neurons in laminae I–VII was determined by averaging the counts made in 20 spinal cord sections for each group.

2.2 Clinical trials

Patients with idiopathic TN received HBO_2 treatment provided by HBO_2 medical device in Xijing Hospital. The clinical trials was conducted according to Declaration of Helsinki principles, approved by the ethics committee of Xijing Hospital (No.200803271) (2008) and registered in the Clinical Trials Registration Center of National Library of the United States (NCT00866424) (2009). A total of 42 patients diagnosed as idiopathic TN were included. These patients were taking carbamazepine (CBZ) treatment for over 1 month with the doses of CBZ required for keeping patient pain at a minimum. The patients were randomly divided into two groups, HBO_2 group ($n = 20$) and HBO_2 sham control group ($n = 22$). Data from three and four other patients in the HBO_2 and the control groups, respectively, were incomplete and thus discarded. The patients in the HBO_2 group received HBO_2 treatment (1.8 ATA, once a day for 10 consecutive days, each for 70 min). The procedure used for HBO_2 treatment in the patients was similar to that previously described in clinical studies (Rossignol et al., 2007; Eschenfelder et al., 2008; Li et al., 2011) and was conducted in accordance with the policy of routine modality of HBO_2 treatment in Xijing Hospital. HBO_2 treatment for the patients was carried out in a multi-place chamber (GY2200, Hongyuan, China). The patients in the HBO_2 group underwent HBO_2 for 70 min daily for 10 consecutive days. Each HBO_2 session sequence was composed of 20 min of slow pressurization from 1 to 1.8 ATA. Once at 2.0 ATA, the patients began to breathe 100% oxygen supplied by a clear plastic hood for two 35-min periods with 5-min interval when the hood was moved away. At the end of the HBO_2 treatment, the chamber was depressurized slowly from 1.8 to 1 ATA over 25 min. The patients breathed only air in the chamber during the period of pressurization, depressurization and interval.

The HBO_2 sham group participants underwent a brief compression to 1.1 ATA at the beginning of each treatment. The chamber was then slowly decompressed from 1.1 to 1.03 ATA where the pressure stayed for the remainder of the treatment. No oxygen was added to the chamber, and thus, the chamber was pressurized with room air (approximately 21% oxygen). The patients in the HBO_2 sham group remained in the chamber for the same length of time as patients in the HBO_2 treatment group. At the end of each treatment, the pressure was slowly increased to 1.1 ATA over about 5 min and then the chamber was depressurized. Procedures were developed and applied to mimic, for the control group, the experience of hyperbaric treatment at 1.1 ATA and thereby to keep the participants unaware of the nature of the intervention. These procedures included covering control switches, inflating and deflating the chambers to simulate pressure changes and masking the sounds from the chambers. A pressure of 1.03 ATA (with increases to 1.1 ATA for several minutes at the beginning and at the end of the treatment) was chosen for the control group because this pressure represented the lowest that could be applied and still effectively simulate hyperbaric treatment at 1.8 ATA. Changes in pain intensity were evaluated by calculating changes of doses of CBZ required for keeping patient pain at a minimum and scores of visual analogue scales (VAS) (Price et al., 1994; Feine et al., 1998), which was used for patient's self-evaluation.

2.3 Statistical analysis

SPSS Rel 15 (SPSS Inc., Chicago, IL, USA) was used to conduct all the statistical analyses. Alteration of expression of the proteins detected and the behavioural responses to thermal and mechanical stimuli over time among groups in animals were tested with one-way and two-way analysis of variance (ANOVA) with repeated measures followed by Bonferroni post hoc tests, respectively. Differences in changes of CBZ doses between the HBO_2 treatment and the control groups were tested with one-way ANOVA. Changes in percentage of patients with significant reduction of severity of pain evaluated by VAS were tested with χ^2 test. Data are presented as mean ± standard error of mean (SEM) or percentage proportion of patients with VAS tests. Statistical results are considered significant if $p < 0.05$.

3. Results

3.1 HBO_2 treatment in early phase after CCI produces a long-lasting inhibition of thermal hyperalgesia and mechanical allodynia

CCI produced rapid-onset and long-lasting thermal hyperalgesia and mechanical allodynia in the rats. HBO_2 treatment (3 ATA, pure oxygen, starting ~30 min after surgery, once a day for seven consecutive days) significantly reduced severity and shortened duration of thermal hyperalgesia and mechanical allodynia. Within the first 2 weeks, latency of foot withdrawal representing thermal sensitivity was reduced approximately 70% (vs. CCI treatment alone). Treatment with HBA (3 ATA, air) or pure oxygen (1 ATA) alone, in the same protocol, did not significantly alter the increased thermal sensitivity. In addition to decreasing severity of pain, HBO_2 treatment shortened duration of the thermal hyperalgesia to 2 weeks from at least 5 weeks in CCI rats (Fig. 1A). HBO_2, but not HBA, and pure oxygen caused analgesic effects on mechanical allodynia (Fig. 1B), which was similar to that on the thermal hyperalgesia. HBA treatment altered neither thermal hyperalgesia nor mechanical allodynia. Treatment with pure oxygen was found to slightly enhance the increased sensitivity to thermal stimulus within 7–10 days after treatment (Fig. 1A and B). These results indicate that HBO_2 therapy (a combination of high pressure and pure oxygen), but not high pressure or pure oxygen alone, applied in the early phase after nerve injury can effectively attenuate severity and shorten duration of neuropathic pain.

Furthermore, we examined how much pressure would be appropriate for producing such an analgesic effect. Three different pressures, 1.5, 2 and 3 ATA, were applied in the same protocol to CCI rats. Pressure at 1.5 and 2 ATA combined with pure oxygen did not significantly alter the pain sensitivity. Pressure at 3 ATA produced significant analgesic effects on the thermal hyperalgesia (Fig. 1C) and mechanical allodynia (Fig. 1D) in CCI rats.

3.2 HBO_2 treatment in later phase after CCI produces a dose-dependent inhibition of thermal hyperalgesia and mechanical allodynia

We continued to examine possible analgesic effects of HBO_2 treatment on ongoing neuropathic hyperalgesia after CCI. Repetitive HBO_2 treatment (3 ATA, each 70 min), starting on the postoperative 14 day, once a day for three consecutive days, produced a significant, transient inhibition of thermal hyperalgesia (Fig. 2A) and mechanical allodynia (Fig. 2B). Inhibition lasted for 5–8 days after termination of the last HBO_2 treatment. Prolonged HBO_2 treatment in the same protocol for seven consecutive days (during postoperative 14–20 days) produced long-lasting inhibition, which sustained the thermal and mechanical hypersensitivity at similar decreased levels for at least 2 weeks after termination of the last treatment (Fig. 2C and D). These results demonstrate that repetitive HBO_2 treatment is also effective in treating the well-developed, persistent pain and prolonged exposure of HBO_2 may result in better treatment effects of the neuropathic pain symptoms.

3.3 HBO_2 treatment suppresses CCI-induced induction of c-Fos, activation of astrocytes and increased phosphorylation of NR2B, ERK, CaMKII and CREB in the spinal cord

CCI caused neurochemical alterations such as induction of c-Fos and activation of astrocytes in the DH ipsilateral to nerve injury, in addition to behavioural signs of neuropathic pain. HBO_2 treatment (3 ATA) applied at the early phase (starting within 30 min after surgery, once a day for seven consecutive days) or at the late phase (starting on 14 postoperative day, once a day for seven consecutive days) significantly inhibited expression of c-Fos (Fig. 3A) and activation of astrocytes (Fig. 3B), respectively. NMDA receptors and the subsequent Ca^{2+}-dependent signals have a well-developed role in neural plasticity and in various pain states. CCI significantly increased levels of pNR1, pNR2B, pERK, pCaMKII and pCREB detected on the

14 postoperative days. Repetitive HBO_2 treatment at the early phase significantly inhibited CCI-induced increased level of pNR2B, pERK, pCaMKII and pCREB (Fig. 4A and C–F), but not the level of pNR1 (Fig. 4A and B). These results indicate that HBO_2 treatment can efficiently reverse the increased neural activities and activities of NR2B receptor and the subsequent Ca^{2+}-dependent signals while attenuating behavioural signs of neuropathic pain.

3.4 HBO_2 treatment attenuates TN in patients

Given that HBO_2 therapy can markedly reduce neuropathic pain and reverse the associated alterations of NMDA receptors and the subsequent Ca^{2+}-dependent signals in rats, we wondered whether HBO_2 therapy could relieve neuropathic pain in patients. We thus applied HBO_2 therapy to patients who were diagnosed with idiopathic TN, a severe form of neuropathic facial pain and there is a lack of high-quality evidence to guide management (Zakrzewska, 2011). Data were analysed from 42 qualified patients. The sample was composed of 19% (8/42) men and 81% (34/42) women. The ages of the participants ranged from 40 to 70, with a mean age of 56.5 ± 7.6 years. The length of time elapsed since first experiencing TN symptoms ranged from 2 to 20 years. The mean length of illness was 14.6 ± 5.1 years. Each of these patients was receiving regular analgesic treatment with CBZ for over 3 months. Effects of HBO_2 therapy on these patients were

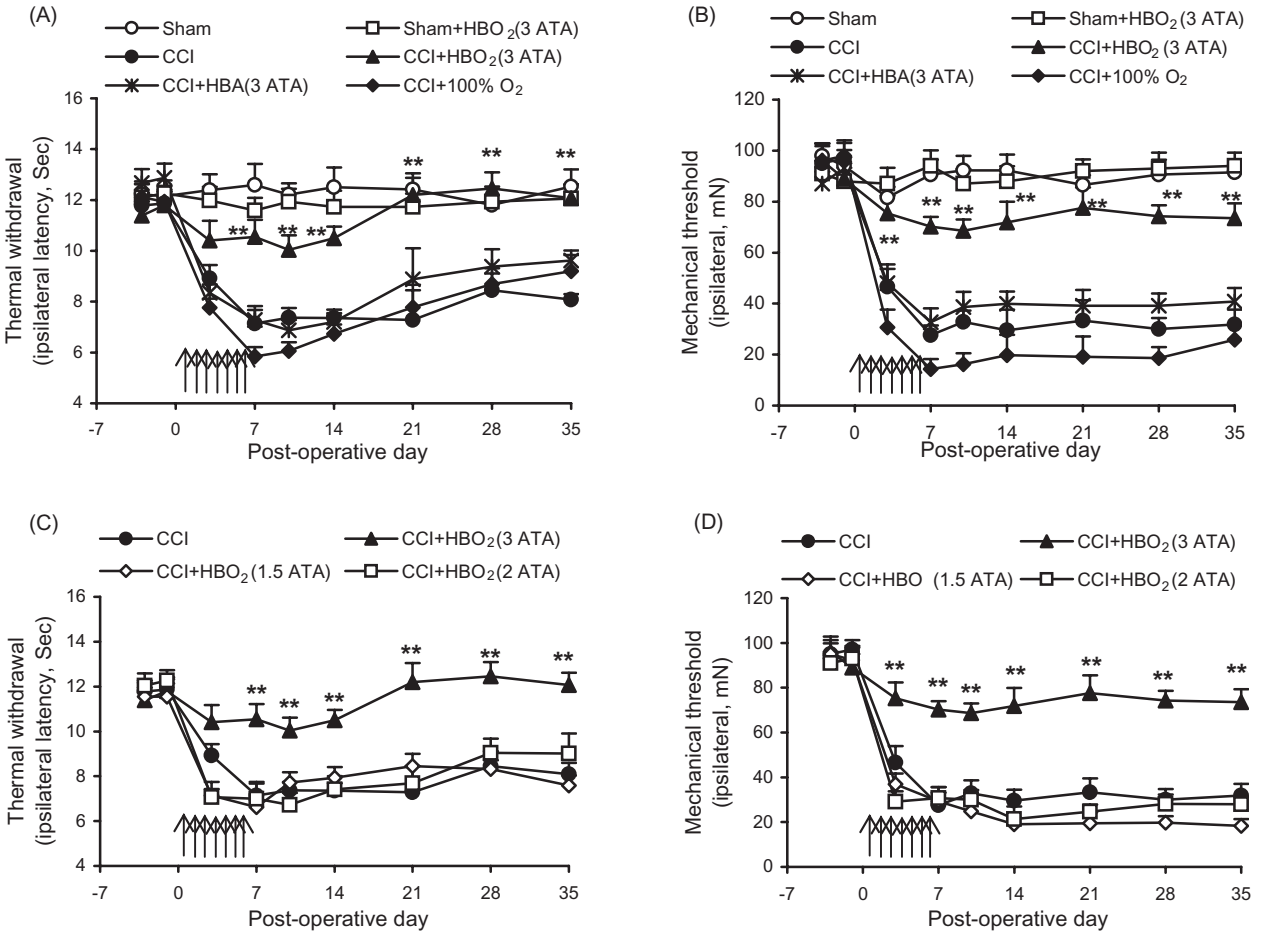

Figure 1 Repetitive HBO_2 treatment in the early phase after nerve injury inhibits production and persistence of thermal hyperalgesia and mechanical allodynia. Repeated measurements are shown of thermal and mechanical sensitivity of the foot-withdrawal responses in CCI- and sham-operated rats. (A and B) Repetitive treatment of HBO_2, HBA or pure oxygen (100% O_2) on thermal hyperalgesia (A) and mechanical allodynia (B). (C, D) Dose-dependent effects of repetitive HBO_2 on thermal hyperalgesia (C) and mechanical allodynia (D). HBO_2, HBA and pure oxygen, respectively, were given at the early phase after CCI (day 0–6). Each group included eight rats. Data represent changes of the withdrawals of the ipsilateral foot. There were no significant changes in the feet contralateral to the injury or in the sham-operated animals (data not shown). An arrow indicates a treatment on the corresponding time point. $*p < 0.05$, $**p < 0.01$ versus CCI group (two-way ANOVA with repeated measures followed by Bonferroni post hoc tests).

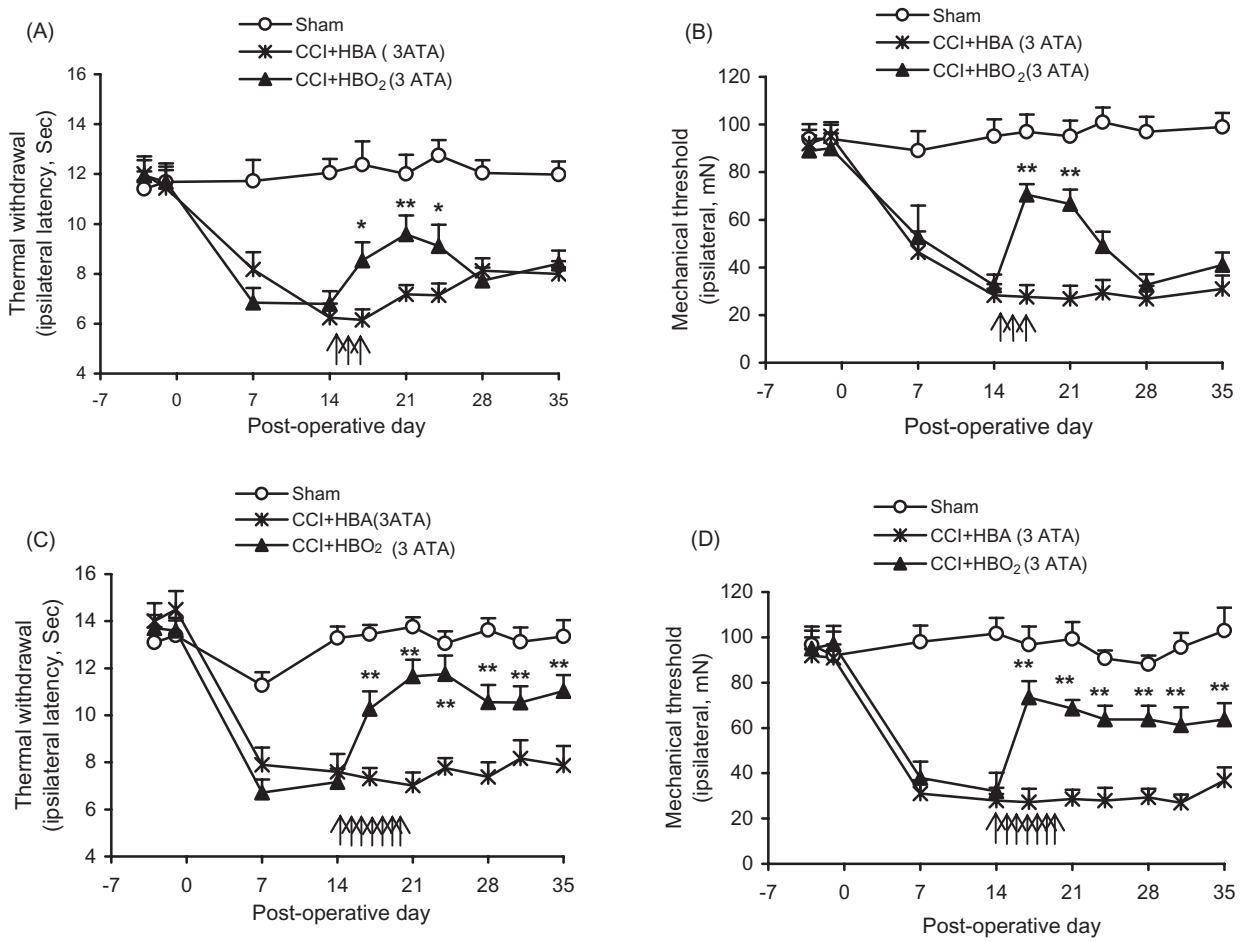

Figure 2 Dose-dependent inhibitory effects of repetitive HBO_2 treatment in the later phase after nerve injury on thermal hyperalgesia and mechanical allodynia. Repeated measurements are shown of thermal and mechanical sensitivity of the foot-withdrawal responses in CCI- and sham-operated rats. (A and B) Three doses of HBO_2, but not HBA, produced a transient inhibition of thermal hyperalgesia (A) and mechanical allodynia (B). (C and D) Seven doses of HBO_2, but not HBA, produced a long-lasting inhibition of thermal hyperalgesia (C) and mechanical allodynia (D). Each group included eight rats. Data represent changes of the withdrawals of the ipsilateral feet. There were no significant changes in the feet contralateral to the injury or in sham-operated animals (data not shown). An arrow indicates a treatment on the corresponding time point. $*p < 0.05$, $**p < 0.01$ versus CCI group (two-way ANOVA with repeated measures followed by Bonferroni post hoc tests).

determined by calculating changes of the daily doses of CBZ required for keeping patient pain at a minimum and the scores of VAS, which was used for patient's self-evaluation.

Following HBO_2 therapy (1.8 ATA, each 70 min, once a day for 10 consecutive days), the required doses of CBZ were significantly reduced on the first evaluation made the next day (day 1) after the last treatment. This analgesic effect lasted for at least 60 days. The CBZ doses returned to the control level 60–90 days after HBO_2 therapy. Data are summarized in Fig. 5A. We noticed that the required dose of CBZ for the patients who received the HBO_2 sham treatment (air, 1 ATA) were also slightly reduced (15–20%) (Fig. 5A). VAS evaluation indicated that HBO2 therapy reduced pain in most patients (60–75%) and such analgesia lasted for about 6 months after the therapy. Again, some patients (20–30%) who received the HBO_2 sham treatment experienced significant reduction of pain syndromes (VAS score decreased > 2) (Fig. 5B), suggesting that psychological effects may contribute to pain relief in these patients but not in the rat (see Figs. 1 and 2). There was no side effects were observed in these patients who received either HBO_2 or HBO_2 sham treatment. This clinical trial indicates that HBO_2 therapy is a safe and effective approach to relieve pain syndromes in most patients suffering idiopathic TN.

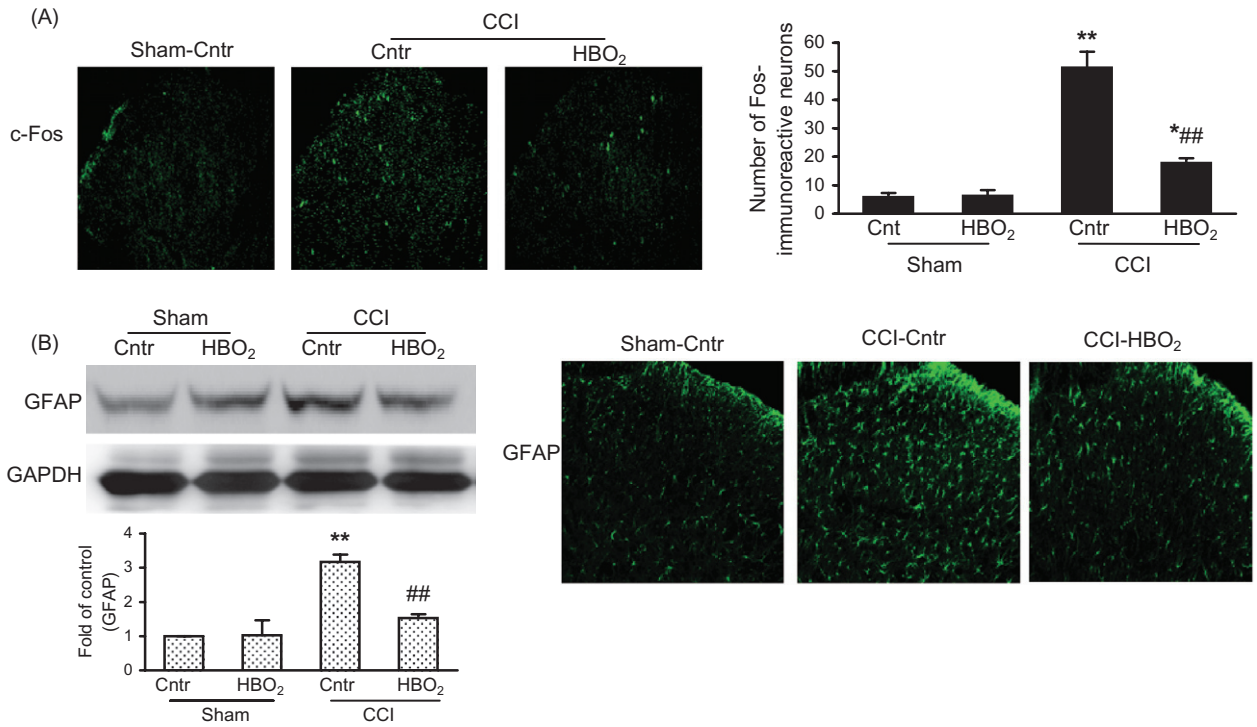

Figure 3 Repetitive HBO_2 treatment suppresses CCI-induced induction of c-Fos and activation of astrocytes in rat spinal cord. (A) Expression of c-Fos in the DH. Examples of induction of c-Fos are shown (left) and data summarized (right). (B) Expression of astrocytes (GFAP). Left: representative western blot bands of GFAP and data summary. Four spinal cord segments were included in each of the groups. Right: examples of confocal images of expression of GFAP in the DH. Tissues were taken on the 14 postoperative day following HBO_2 treatment (seven doses during 0–6 postoperative day). $*p < 0.05$, $**p < 0.01$ versus corresponding control (Cntr) group in sham-operated animals (A); $\#\#p < 0.01$ versus corresponding Cntr group in CCI animals (B). Magnification: 200×.

4. Discussion and conclusion

Our study demonstrates that HBO_2 therapy can significantly attenuate neuropathic pain in animals with peripheral nerve injury and in patients with idiopathic TN. HBO_2 therapy produces a rapid-onset, dose-dependent and long-lasting inhibition of thermal hyperalgesia and mechanical allodynia and the associated c-Fos induction and astrocyte activation as well as the increased phosphorylation of NR2B receptor and the subsequent Ca^{2+}-dependent signals in rats after nerve injury. In the clinical trial, one course of HBO_2 therapy (10 consecutive days) results in a rapid-onset and long-lasting analgesic effects evidenced by the significantly decreased doses of CBZ required for keeping patient pain at a minimum and decreased scores of VAS. This study strongly supports that HBO_2 therapy is an effective and safe approach for reducing neuropathic pain in animals and in humans.

HBO_2 therapy has been clinically used for the protection of the nervous system after acute injury, such as stroke (Zhang et al., 2005) and hippocampus ischaemia (Wada et al., 1996) as well as improving peripheral nerve repair and regeneration (Tibbles and Edelsberg, 1996; Sanchez, 2007). HBO_2 preconditioning can induce tolerance against spinal cord ischaemia (Dong et al., 2002; Nie et al., 2006). Recently, HBO_2 therapy is reported to be effective in treating headache (Di Sabato et al., 1993) and complex regional pain syndrome (Kiralp et al., 2004) in patients and attenuating inflammatory pain in animals (Sumen et al., 2001; Wilson et al., 2007; Zelinski et al., 2009). These findings support an important role of HBO_2 in neural protection and anti-inflammation. Here, we show that HBO_2 therapy can markedly attenuate neuropathic pain in animals after sciatic nerve injury and in patients with idiopathic TN. In the clinical trials, we realized that the HBO_2 sham group patients should have been subjected to 1.8 ATA room air so that patients feeling the increased pressure will not be biased in their self-evaluation that they should be feeling less pain and therefore require less CBZ. This possibility might have been largely diminished since

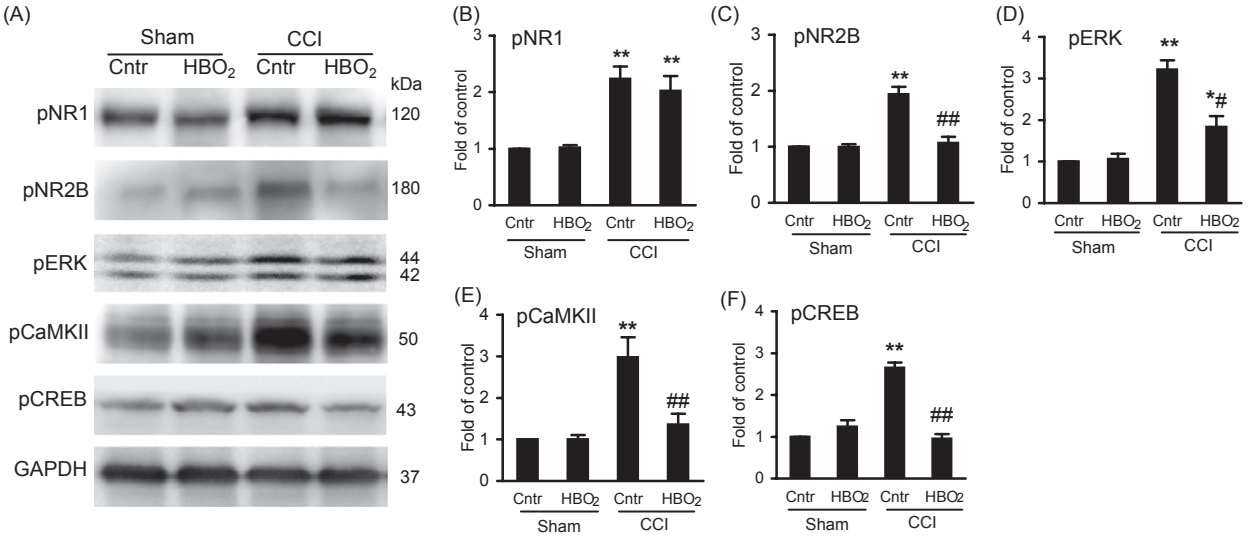

Figure 4 Repetitive HBO_2 treatment suppresses CCI-induced increased phosphorylation of NR2B, ERK, CaMKII and CREB in the spinal cord. (A) Representative western blot bands of pNR1, pNR2B, pERK, pCaMKII and pCREB. (B–F) Data summary. Four spinal cord segments were included in each of the groups. Tissues were taken on the 14 postoperative day following HBO_2 treatment (seven doses during 0–6 postoperative day). $*p < 0.05$, $**p < 0.01$ versus corresponding control (Cntr) group in sham-operated animals; $\#p < 0.05$, $\#\#p < 0.01$ versus corresponding Cntr group in CCI animals.

the best blinding control was applied to the HBO_2 sham group patients (see methods section).

This is supported by a recent study in which suppression of mechanical allodynia in rats after nerve injury was briefly described (Thompson et al., 2010). We further show that HBO_2 therapy can produce a rapid-onset, dose-dependent and long-lasting analgesic effect. It is interesting that neither high pressure nor pure oxygen alone results in an analgesic effect, i.e., a combination of high pressure and pure oxygen is required. In the experimental rats, repetitive HBO_2 treatment produced a significant, transient inhibition of thermal hyperalgesia (Fig. 2A) and mechanical allodynia (Fig. 2B). Inhibition lasted for 5–8 days after termination of the last HBO_2 treatment. Prolonged HBO_2 treatment produced long-lasting inhibition, which sustained the thermal and mechanical hypersensitivity at similar decreased levels for at least 2 weeks after termination of the last treatment (Fig. 2C and D). These results suggest that more intermittent regimen of HBO_2 treatment might have produced a more sustained time of suppressing neuropathic pain. In rats, repetitive HBO_2 therapy with 3 ATA plus pure oxygen is required to produce analgesia, while in the patients with idiopathic TN, HBO_2 therapy with 1.8 ATA plus pure oxygen can markedly reduce severity of pain. We did not apply higher pressure to the patients because it was prohibited by the policy of the Xijing Hospital. Relative lower pressure is required in humans than in rats. The possible reasons are unknown and were not examined in this study. We wondered that the humans would be more sensitive to HBO_2 than rats because the humans' body weight and size are hundreds of times greater than rats', meanwhile the rats' metabolic rate may be higher than the humans. Another reason might be that the pain evaluation system for the humans is more accurate than that for rats. The patients could use language to describe their feelings and communicate with doctors, in addition to the objective methods used including the use of CBZ as well as VAS (Price et al., 1994; Feine et al., 1998). Thus, the patients are judged more sensitive to the HBO_2 treatment. However, the pain experienced by the rats was described by researchers based only on the measurements of the mechanical withdrawal threshold and thermal withdrawal latency of the feet tested. HBO_2 therapy with 1.8 ATA can produce a good analgesic effect in most evaluated patients in our study. It is unclear whether HBO_2 with higher pressure (e.g., 2–3 ATA) may result in better treatment effect. We noticed that some studies have shown that HBO_2 treatment with higher pressure in the range of 2.4–3 ATA can produce better treatment effects in patients (Holbach et al., 1977; Nighoghossian et al., 1995). However, although it is agreed that the use of HBO_2 treatment is relatively safe, the possible side effects need to be known by the patients. In this study, several patients felt mild uncomfortable in their ears (probably caused

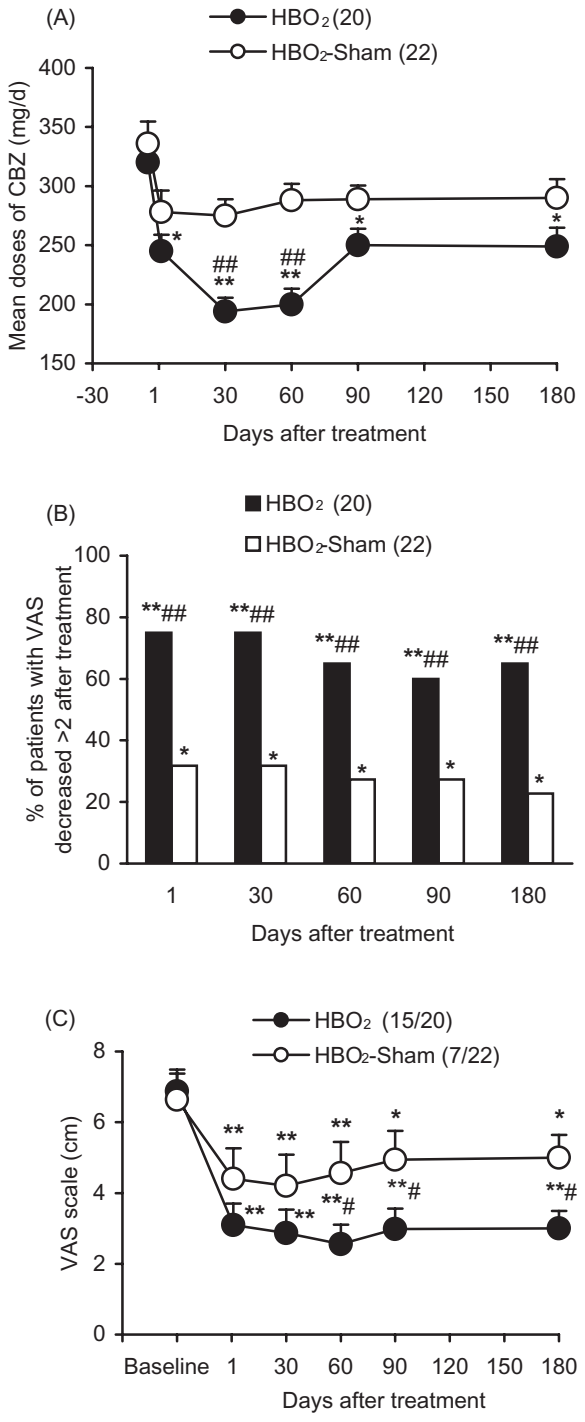

Figure 5 HBO_2 treatment attenuates pain in patients with idiopathic TN. (A) Decreases of the mean doses of CBZ requirement for keeping patient pain at a minimum. The patients received treatment of HBO_2 (1.8 ATA, 100%O2) or sham treatment (HBO_2 sham, 1 ATA, air), each for 70 min, once a day for 10 consecutive days. (B) Percentage of patients with significant reduction of severity of pain evaluated by VAS (VAS decreased >2) after treatment of HBO_2 and HBO_2 sham, respectively. $*p < 0.05$, $**p < 0.01$ versus pretreatment within the group; $\#p < 0.05$, $\#\#p < 0.01$ versus HBO_2 sham group.

by poor Eustachian tube opening because of the pressure) when they were receiving HBO_2 treatment, but they all agreed that the slight uncomfortable feeling was tolerable and could be relieved or reduced by swallowing, chewing, etc.

HBO_2 therapy may protect the injured nerve tissues through reducing tissue oedema and hypoxia, improving microcirculation and enhancing regeneration of micro-capillaries and the nerve tissues, inhibiting inflammatory responses (Tibbles and Edelsberg, 1996; Waisman et al., 2003; Sanchez, 2007) and inducing adaptive protection against oxidative stress (Rothfuss and Speit, 2002). Neuropathic pain is initially triggered by nerve damage. The damaged nerves and their neighbouring neurons and non-neural cells are observed to have inflammation and de-myelination in DRG. Thus, we hypothesize that mechanisms underlying the analgesic effects produced by HBO_2 therapy may include minimizing and protecting the damaged nerve from being further damaged and reversing or interrupting the injury-induced processing of nociception and inhibiting inflammatory responses secondary to the nerve injury. Our results that HBO_2 treatment can inhibit CCI-induced activation of astrocytes supports the possibility of inhibition of inflammation since activated astrocytes can release pro-inflammatory cytokines (e.g., interleukin-1β) and chemokines (e.g., monocyte chemoattractant protein-1) in the spinal cord to enhance and prolong persistent pain states (Gao and Ji, 2010). The earlier the HBO_2 therapy applied and inflammation inhibited, the better the analgesic effects achieved (comparing the treatment effects shown in Figs. 1 and 2). NMDA receptors and the subsequent Ca^{2+}-dependent signals have a well-developed role in neural plasticity and in neuropathic pain states. Our results show that HBO_2 treatment can markedly suppress the nerve injury-induced increased level of phosphorylation of NR2B, ERK, CaMKII and CREB and induction of c-Fos. This suggests that HBO_2 therapy may also reduce neuropathic pain through inhibition of these pain signalling pathways. It is also possible that inhibition of these signals is secondary to the analgesic effects and/or inhibition of the inflammatory responses, etc.

In conclusion, we have demonstrated that HBO_2 therapy is an effective and safe approach to relieve neuropathic pain in rats after peripheral nerve injury and in patients suffering idiopathic TN. Neural protection, anti-inflammation and inhibition of nerve injury-induced altered neural activity may contribute to the analgesic effect of HBO_2 therapy.

Author contributions

N. G., W.-T.L. and S.L. performed the animal behavioural studies and analysed data; J.-Y.N., Y.-Y.S, Y. L and H.-L.D. conducted the clinical trials and analysed data. X.-J.S. designed and supervised the animal studies and wrote the manuscript. L.X. supervised the clinica trials. All authors discussed the results and commented on the manuscript.

References

Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 1988;33:87–107.

Di Sabato F, Fusco BM, Pelaia P, Giacovazzo M. Hyperbaric oxygen therapy in cluster headache. Pain 1993;52:243–5.

Dong H, Xiong L, Zhu Z, Chen S, Hou L, Sakabe T. Preconditioning with hyperbaric oxygen and hyperoxia induces tolerance against spinal cord ischemia in rabbits. Anesthesiology 2002;96:907–12.

Eschenfelder CC, Krug R, Yusofi AF, Meyne JK, Herdegen T, Koch A, et al. Neuroprotection by oxygen in acute transient focal cerebral ischemia is dose dependent and shows superiority of hyperbaric oxygenation. Cerebrovasc Dis 2008; 25:193–201.

Feine JS, Lavigne GJ, Dao TT, Morin C, Lund JP. Memories of chronic pain and perceptions of relief. Pain 1998;77:137–41.

Gao YJ, Ji RR. Targeting astrocyte signaling for chronic pain. Neurotherapeutics 2010;7:482–93.

Holbach KH, Caroli A, Wassmann H. Cerebral energy metabolism in patients with brain lesions of normo- and hyperbaric oxygen pressures. J Neurol 1977;217:17–30.

Ikeda H, Heinke B, Ruscheweyh R, Sandkuhler J. Synaptic plasticity in spinal lamina I projection neurons that mediate hyperalgesia. Science 2003;299:1237–40.

Kiralp MZ, Yildiz S, Vural D, Keskin I, Ay H, Dursun H. Effectiveness of hyperbaric oxygen therapy in the treatment of complex regional pain syndrome. J Int Med Res 2004;32:258–62.

Kohno T, Moore KA, Baba H, Woolf CJ. Peripheral nerve injury alters excitatory synaptic transmission in lamina II of the rat dorsal horn. J Physiol 2003;548:131–8.

Krafft RM. Trigeminal neuralgia. Am Fam Physician 2008; 77:1291–6.

Li Y, Dong H, Chen M, Liu J, Yang L, Chen S, et al. Preconditioning with repeated hyperbaric oxygen induces myocardial and cerebral protection in patients undergoing coronary artery bypass graft surgery: a prospective, randomized, controlled clinical trial. J Cardiothorac Vasc Anesth 2011;25:908–16.

Liu WT, Han Y, Liu YP, Song AA, Barnes B, Song XJ. Spinal matrix metalloproteinase-9 contributes to physical dependence on morphine in mice. J Neurosci 2010;30:7613–23.

Moore KA, Kohno T, Karchewski LA, Scholz J, Baba H, Woolf CJ. Partial peripheral nerve injury promotes a selective loss of GABAergic inhibition in the superficial dorsal horn of the spinal cord. J Neurosci 2002;22:6724–31.

Nie H, Xiong L, Lao N, Chen S, Xu N, Zhu Z. Hyperbaric oxygen preconditioning induces tolerance against spinal cord ischemia by upregulation of antioxidant enzymes in rabbits. J Cereb Blood Flow Metab 2006;26:666–74.

Nighoghossian N, Trouillas P, Adeleine P, Salord F. Hyperbaric oxygen in the treatment of acute ischemic stroke. A double-blind pilot study. Stroke 1995;26:1369–72.

Price DD, Bush FM, Long S, Harkins SW. A comparison of pain measurement characteristics of mechanical visual analogue and simple numerical rating scales. Pain 1994;56:217–26.

Rossignol DA, Rossignol LW, James SJ, Melnyk S, Mumper E. The effects of hyperbaric oxygen therapy on oxidative stress, inflammation, and symptoms in children with autism: an open-label pilot study. BMC Pediatr 2007;7:36.

Rothfuss A, Speit G. Investigations on the mechanism of hyperbaric oxygen (HBO)-induced adaptive protection against oxidative stress. Mutat Res 2002;508:157–65.

Sanchez EC. Hyperbaric oxygenation in peripheral nerve repair and regeneration. Neurol Res 2007;29:184–98.

Song XJ, Hu SJ, Greenquist KW, Zhang JM, LaMotte RH. Mechanical and thermal hyperalgesia and ectopic neuronal discharge after chronic compression of dorsal root ganglia. J Neurophysiol 1999;82:3347–58.

Song XJ, Vizcarra C, Xu DS, Rupert RL, Wong ZN. Hyperalgesia and neural excitability following injuries to central and peripheral branches of axons and somata of dorsal root ganglion neurons. J Neurophysiol 2003;89:2185–93.

Song XJ, Wang ZB, Gan Q, Walters ET. cAMP and cGMP contribute to sensory neuron hyperexcitability and hyperalgesia in rats with dorsal root ganglia compression. J Neurophysiol 2006;95:479–92.

Song XJ, Zheng JH, Cao JL, Liu WT, Song XS, Huang ZJ. EphrinB-EphB receptor signaling contributes to neuropathic pain by regulating neural excitability and spinal synaptic plasticity in rats. Pain 2008;139:168–80.

Sumen G, Cimsit M, Eroglu L. Hyperbaric oxygen treatment reduces carrageenan-induced acute inflammation in rats. Eur J Pharmacol 2001;431:265–8.

Thompson CD, Uhelski ML, Wilson JR, Fuchs PN. Hyperbaric oxygen treatment decreases pain in two nerve injury models. Neurosci Res 2010;66:279–83.

Tibbles PM, Edelsberg JS. Hyperbaric-oxygen therapy. N Engl J Med 1996;334:1642–8.

Wada K, Ito M, Miyazawa T, Katoh H, Nawashiro H, Shima K, et al. Repeated hyperbaric oxygen induces ischemic tolerance in gerbil hippocampus. Brain Res 1996;740:15–20.

Waisman D, Brod V, Wolff R, Sabo E, Chernin M, Weintraub Z, et al. Effects of hyperoxia on local and remote microcirculatory inflammatory response after splanchnic ischemia and reperfusion. Am J Physiol Heart Circ Physiol 2003;285:H643–52.

Wang ZB, Gan Q, Rupert RL, Zeng YM, Song XJ. Thiamine, pyridoxine, cyanocobalamin and their combination inhibit thermal, but not mechanical hyperalgesia in rats with primary sensory neuron injury. Pain 2005;114:266–77.

Wilson HD, Toepfer VE, Senapati AK, Wilson JR, Fuchs PN. Hyperbaric oxygen treatment is comparable to acetylsalicylic acid treatment in an animal model of arthritis. J Pain 2007; 8:924–30.

Wilson HD, Wilson JR, Fuchs PN. Hyperbaric oxygen treatment decreases inflammation and mechanical hypersensitivity in an animal model of inflammatory pain. Brain Res 2006; 1098:126–8.

Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. Pain 2010;152:S2–15.

Xiong L, Zhu Z, Dong H, Hu W, Hou L, Chen S. Hyperbaric oxygen preconditioning induces neuroprotection against

ischemia in transient not permanent middle cerebral artery occlusion rat model. Chin Med J (Engl) 2000;113:836–9.
Yamashita S, Hirata T, Mizukami Y, Cui YJ, Fukuda S, Ishida K, et al. Repeated preconditioning with hyperbaric oxygen induces neuroprotection against forebrain ischemia via suppression of p38 mitogen activated protein kinase. Brain Res 2009;1301:171–9.
Zakrzewska JM. Surgery for trigeminal neuralgia? Pain 2011;152:469–70.
Zelinski LM, Ohgami Y, Chung E, Shirachi DY, Quock RM. A prolonged nitric oxide-dependent, opioid-mediated antinociceptive effect of hyperbaric oxygen in mice. J Pain 2009; 10:167–72.
Zhang JH, Lo T, Mychaskiw G, Colohan A. Mechanisms of hyperbaric oxygen and neuroprotection in stroke. Pathophysiology 2005;12:63–77.
Zheng JH, Walters ET, Song XJ. Dissociation of dorsal root ganglion neurons induces hyperexcitability that is maintained by increased responsiveness to cAMP and cGMP. J Neurophysiol 2007;97:15–25.