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Hyperbaric oxygen therapy and coenzyme Q10 synergistically attenuates damage progression in spinal cord injury in a rat model

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ABSTRACT

Background: Identifying effective spinal cord injury (SCI) treatments remains a major challenge, and current approaches are still unable to effectively improve its. Currently, we investigated the combined effects of hyperbaric oxygen (HBO) along with coenzyme Q10 (CoQ10) in the recovery of SCI in rats.

Material and methods: Ninety female mature Sprague-Dawley rats were allocated into five equal groups, including; sham group, SCI group, HBO group (underwent SCI and received HBO), CoQ10 group (underwent SCI and received CoQ10), and HBO+CoQ10 group (underwent SCI and received HBO plus CoQ10). Tissue samples at the lesion site were obtained for evaluation of stereological, immunohistochemical, biochemical, molecular. Also, functional tests were performed to evaluate of behavioral properties.

Results: We found that a significant increase in stereological parameters, biochemical factors (GSH, SOD and CAT), IL-10 gene expression and behavioral functions (BBB and EMG Latency) in the treatment groups, especially HBO+CoQ10 group, compared to SCI group. In addition, MDA levels, the density of apoptotic cells, as well as expression of inflammatory genes (TNF-α and IL-1β) were considerably reduced in the treatment groups, especially HBO+CoQ10 group, compared to SCI group.

Conclusion: We conclude that co-administration of HBO and HBO+CoQ10 has a synergistic neuroprotective effects in animals undergoing SCI.

1. Introduction

Trauma-induced spinal cord injury (SCI) is one of the most common neurological disorders and imposes a significant economic burden on patients and society. Much effort has been expended to find effective treatments for SCI, but current treatments need improvement (Alizadeh et al., 2019). In SCI, a cascade of molecular and cellular events begins after trauma that can lead to destructive events such as apoptosis, gliosis, excessive inflammation and oxidative stress at the injury site (Mirzaie et al., 2022). Therefore, this is the secondary post-traumatic

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stage that can lead to escalation of injury and loss of sensory and motor pathways at the site of injury and the underlying spinal cord (Cheshmi et al., 2023). Studies have shown that the most effective intervention in spinal cord injury is to perform rapid treatment measures and prevent the spread of secondary damage after trauma (Anjum et al., 2020; David et al., 2019). Despite major advances in the treatment and care of these patients, research into new and effective treatment protocols remains one of the priorities of healthcare systems worldwide (Karsy and Hawryluk, 2019).

Considering that methylprednisolone is the only clinically effective drug in the treatment of SCI patients, it has not been approved in recent years due to significant side effects such as the increased possibility of gastrointestinal bleeding and the subsequent increase in mortality (Liu et al., 2019).

Considering the complexity of the pathological events that occur in SCI, it seems that the most effective treatment is the use of multifactorial approaches that can both inhibit the spread of damage and cause the damaged area to heal.

Hyperbaric oxygen (HBO) is the administration of pure oxygen (100%) at pressures above 1 atmosphere (Izanlu et al., 2022). Currently, this combination is used in the clinic to treat various diseases, including venous ulcers (Armand et al., 2012), diabetic foot's ulcers (Lalieu et al., 2022), spinal cord injury (Sun et al., 2019), decompression sickness, selected crush injuries, compartment syndromes, and other acute traumatic ischemia (Kirby et al., 2019). HBO has high anti-inflammatory, anti-apoptotic and antioxidant properties, and studies have shown that its administration in SCI can significantly prevent the spread of damage (Ahmadi et al., 2022; Huang et al., 2021). We previously reported that the administration of HBO together with methylprednisolone synergistically improves the histological and functional condition of the spinal cord in SCI rats (Ahmadi et al., 2022). Therefore, we have presently investigated the HBO therapy alongside coenzyme Q10 (CoQ10) that some experimental studies reported their beneficial effects in SCI.

CoQ10 is a vitamin-like benzoquinone compound. CoQ10 has an important role in the production of ATP and in the mitochondrial respiratory chain (Raizner, 2019). Studies have shown that CoQ10 has antioxidant and anti-apoptotic properties and its administration in various diseases such as cerebral ischemia (Lu et al., 2017), heart failure (Madmani et al., 2014), hepatotoxicity (Samimi et al., 2019), and reproductive system (Saha et al., 2019) can prevent the progression of damage. Recently, studies have reported the neuroprotective effects of CoQ10 in spinal cord injury (Li et al., 2019; Zhang et al., 2015).

Presently, we investigated whether administration of HBO together with CoQ10 could synergistically prevent damage progression and induce functional improvement in an experimental animal model of SCI.

2. Material and methods

2.1. Experimental spinal cord injury model

In the present study, 90 mature female Sprague-Dawley rats (200–250 g) were recruited. In order to induce SCI, Allen's method was used with minor modification (Mirzaie et al., 2022). Briefly, the rats were anesthetized with intraperitoneal (ip) injection of xylazine (10 mg/kg) and Ketamine (80 mg/kg). Under aseptic conditions, a skin incision was first made between the T6 to T10 vertebrae, followed by a laminectomy on the T9 vertebra. After the spinal cord was exposed, a 100 mg weight was dropped from a height of 5 cm to inflict a contusion. After causing the injury, the skin of the injured area was sutured, and then immediately Cefazolin (Loghman Co, Tehran, Iran) was administered (50 μg/kg; ip) in order to prevent infection (Ahmadi et al., 2022).

The animals were randomly allocated into five equal groups (n = 18). In addition to sham group that only underwent laminectomy, SCI rats were divided into four groups as follows: SCI group; HBO group that received HBO; CoQ10 group that received CoQ10; and HBO+CoQ10 group that simultaneously received HBO and CoQ10.

Experimental studies have shown that the optimal time to study factors influencing the spread of secondary damage in SCI, such as inflammation, cell apoptosis, and oxidative stress, is forty-eight hours after the injury (Christie et al., 2008; Citron et al., 2000). However, neurological examination requires more time (Ahmadi et al., 2022). Therefore, in the current study, treatment assessments were performed in 2 intervals. Forty-eight hours after surgery, 12 rats from each group were sampled for immunohistochemical, molecular and biochemical evaluations. The remaining 6 rats in each group were sampled for stereological evaluations on day 14 after behavioral function testing, as described in Fig. 1. To prevent the possible bias in the results, all evaluations and data analysis were performed by a person who did not have information about the groups and treatments received.

2.2. HBO therapy and CoQ10 administration

A standard hyperbaric oxygen chamber was used for HBO therapy (Ahmadi et al., 2022). The duration of oxygen therapy was 90 min, during which the rats were placed within the chamber and oxygen administration was done in three stages. In the first 15 min, the pressure was gradually increased to avoid damage caused by a sudden increase in pressure. After stabilizing the pressure at 2.5 atmospheres, this pressure was maintained for one hour. And finally, in the last 15 min, the pressure was reduced gradually until it reached 1 atmosphere and the animals were taken out of the chamber. Oxygen was administered three times, including 6, 24, and 48 h after the injury. In the groups receiving CoQ10, immediately after SCI, CoQ10 (Sigma chemicals, St Louis, MO, USA) was administered as a single dose of 20 mg/kg intraperitoneally (ip) and then continued daily for 2 days at the same times (Li et al., 2019).

2.3. Histopathological and stereological evaluations

Tissue samples at the site of the lesion were taken at the end of the study (day 14) and fixed in 10% formalin. Next, the samples were embedded in paraffin and cut using a microtome after routine histological procedures. Ten equally spaced sections were selected from each rat and stained with H&E. The used sections were in two thicknesses. A 5 μm thickness was used to measure the total volumes of the spinal cord and central cavity, and a 20 μm thickness was used to count the numerical densities of neurons and glial cells (Howard and Reed, 2004).

2.3.1. Stereological determination of volume

In order to measure the total volumes of the spinal cord and the central cavity at the site of the lesion, stereological evaluation was performed based on the Cavalieri method. In this method, the image of the tissue sections were superimposed on the grid of points and the points that were placed in the tissue area were counted. After counting the points, total volumes were calculated according as follows: $V_{total} = \sum P \times \frac{a}{p} \times t$: that ΣP was counted points; a/p was the area of each point (mm^2), and t was the distance between two consecutive sections (mm) (Supplementary Figure 1).

2.3.2. Stereological evaluation of the numerical densities of neuron and glial cells

Tissue sections were placed at magnification × 400, then neuron and glial cells that placed within the probe were counted and numerical densities (N_V) were calculated as follows: $N_v = \frac{\sum Q}{\sum P \times h \times \frac{a}{f}} \times \frac{t}{BA}$, where ΣQ was the total number of counted frames; h (μm) was the dissector height; Σp was the total number of counted cells within the probe; a/f (mm^2) was the probe area; BA (μm) was the microtome block advance that set at ten μm; and t (μm) was section thickness (Supplementary Figure 1).

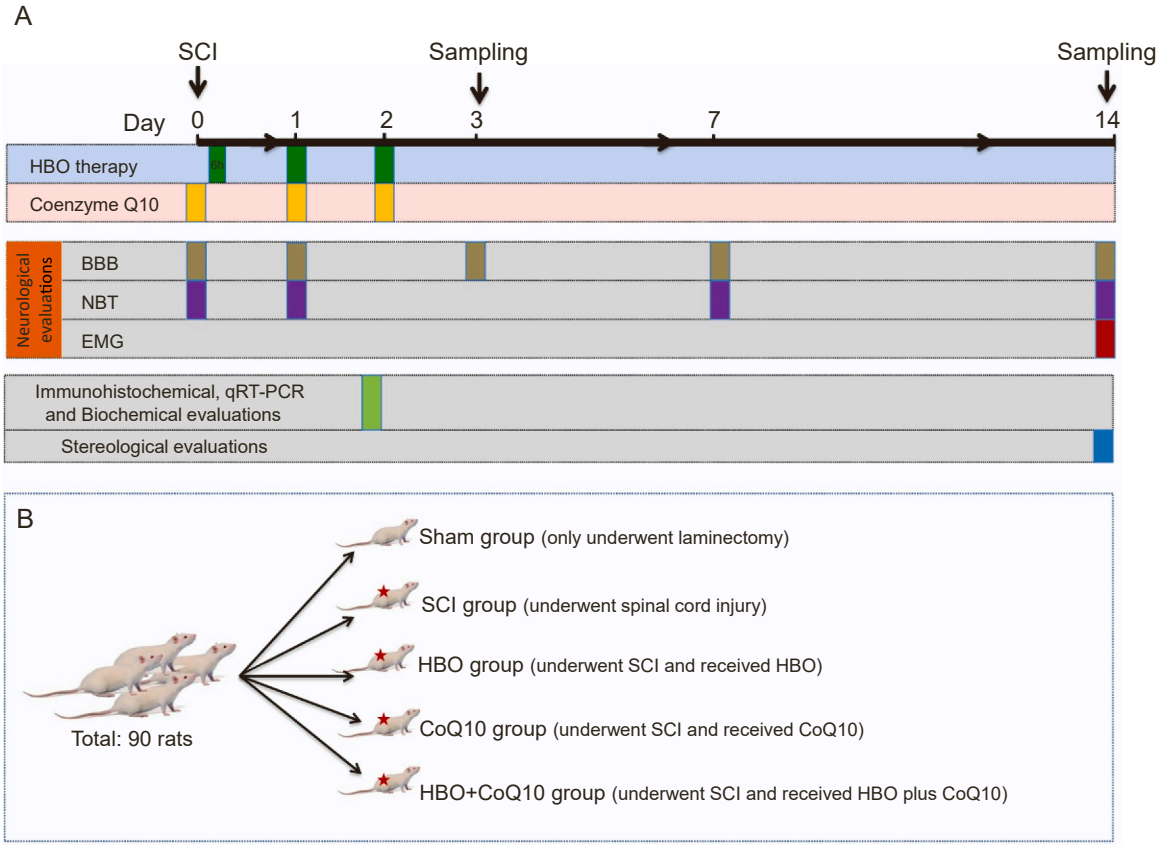

Fig. 1. Scheme of the experimental design. (A) To induce SCI, Allen's method was applied on the T9 level. HBO was administered three times, including 6, 24, and 48 h after the injury. In the groups receiving CoQ10, immediately after SCI, CoQ10 was administered as a single dose of 20 mg/kg intraperitoneally and then continued daily for 2 days at the same times. Sampling was done in 2 time points. Forty-eight hours after surgery, 12 rats from each group were sampled for immunohistochemical, qRT-PCR, and biochemical evaluations. The remaining 6 rats in each group were sampled for stereological evaluations on day 14. (B) Ninety rats were randomly divided into five groups (n = 18). In addition to the sham group that only underwent laminectomy, SCI rats were divided into four groups as follows: SCI group; HBO group; CoQ10 group; and HBO+CoQ10 group (The red star indicates SCI in the studied groups).

2.4. Immunohistochemistry

For determination of apoptosis, immunostaining for caspase-3 antibody was performed. For this purpose, 10 sections with equal intervals from each rat were selected. After deparaffinization, all sections were exposed to goat serum in order to block non-specific sites. Then, Caspase-3 (1:100 in PBS, Abcam ab4250) antibody was added to the sections and incubated overnight at 4 °C. Next, sections were washed with PBS and secondary antibody (Abcam ab97047) was added. Finally, to detection of positive reactions, diaminobenzidine tetrahydrochloride was added. A densitometry method was used to determine the density of positive reactions (Ahmadi et al., 2022).

2.5. Biochemical evaluations

After washing the harvested samples with sterile PBS to remove excess tissue residues, they were immediately frozen at − 80 °C for further analysis. To assess the biochemical status at the site of injury, concentrations of glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT), as antioxidant biomarkers, and malondialdehyde (MDA), as oxidant factor, has been investigated.

The levels of GSH were measured as described by Ellman (1959). For this purpose, the tissue homogenates were mixed with 5% trichloroacetic acid (TCA). The contents were mixed well and 1.0 ml of Ellman's reagent and 0.3 M disodium hydrogen phosphate were added. The absorbance was read at 420 nm against a blank containing TCA. The amount of glutathione is expressed as μmol/mg of protein.

The activity of SOD was measured as described by Misra and Fridovich (1972). Briefly, ethanol and chloroform were added to tissue homogenates followed by adding 0.6 mM EDTA solution and 0.1 M carbonate-bicarbonate buffer. Then, 1.8 mM epinephrine was added to initiate the reaction and absorbance was read at 480 nm. The enzyme activity is expressed as equiv/mg protein.

The CAT activity was measured as described by Aebi (1974). Briefly, 50 mM phosphate buffer was added to the tissue homogenate and the reaction was started by the addition of 30 mM H_2O_2 solution. The absorbance was read at 245 nm. The enzyme activity is expressed as equiv/mg protein.

Finally, the levels of MDA were measured by the method of Uchiyama and Mihara (1978). Briefly, TCA and thiobarbituric acid (TBA) were added to the tissue homogenates and their absorbance was measured at 532 nm. The level of MDA was expressed as μmol/mg of protein.

2.6. Gene expression evaluation

Quantitative real-time PCR (qRT-PCR) method was used to measure IL-10 gene expression as an effective cytokine in immunoregulatory, and TNF-α and IL-1β genes as indicators of inflammation. In this method, harvested tissue samples were homogenized immediately after collection and total RNA was extracted using the Total RNA Extraction Kit (pars-tous). In order to prepare cDNA, cDNA MultiScribe™ kit (ThermoFisher) was used (Cheshmi et al., 2023). The results of cDNA evaluation using spectrophotometry showed that the absorbance ratio for all samples between two wavelengths of 260 and 280 nm were about 1.8–2.01. Finally, gene expression levels were determined using a

real-time PCR instrument (Applied Biosystems StepOne). The sequences of the primers used are shown in Table 1. Additionally, β-actin was used as an internal control.

2.7. Neurological functions

2.7.1. Basso-Beattie-Bresnehan test

The Basso-Beattie-Bresnahan (BBB) locomotor rating scale (Basso et al., 1996) was used to evaluate the recovery of hind limb motor function. For this purpose, the animals were placed within the open-field chamber for five minutes and their motor performance was analyzed. The lowest and highest scores for this test were 0 and 21, respectively, with the maximum scores indicating improved motor status and the minimum scores indicating the severity of post-injury paralysis. Assessments were made before (day 0) and on days 1, 3, 7, and 14 after surgery.

2.7.2. Narrow beam walking test

The narrow beam walking test (NBT) was performed to assess sensory-motor coordination according to the descriptions of von Euler et al. (1996). For this purpose, the animals were placed on a wooden board with a length of 80 cm and a width of 4 cm, and the distance traveled by them was recorded and scored. The evaluated time periods were days 0, 1, 7 and 14 after SCI.

2.7.3. Electromyography test

To measures muscle response to a nerve's stimulation, electromyography (EMG Latency) test was performed (Keller et al., 2018). For this purpose, after anesthetizing the rats, the sciatic nerve was exposed. Then, the stimulating electrode was connected to the upper part of the nerve (near the gluteal muscles) and the receiving electrode was connected to the gastrocnemius muscle. After nerve stimulation, muscle activation rate was recorded and EMG latency was calculated based on milliseconds.

2.8. Statistical analysis

All data were analyzed with SPSS software (v. 21). One-way analysis of variance (ANOVA) with Tukey's post hoc test was used to test relationships between groups. Results are expressed as mean ± standard deviation (SD). In addition, the inter- and within-test coefficients of variation (CV) were also reported for each of the biochemical methods. P-values < 0.05 were considered significant.

3. Results

3.1. Combination of HBO therapy and CoQ10 administration improved the stereological characterizes after SCI

To investigate the amount of stereological parameters, H&E staining was performed (Fig. 2A). Stereological results are shown in Fig. 2B-E. As it is clear in all the graphs, sham group was better in comparison with other groups. Therefore, in the results, only the comparison of other groups has been done.

3.1.1. The total volumes of the spinal cord and central cavity

In evaluation of the total volume of the spinal cord at the lesion site, significantly higher volume was observed in HBO, CoQ10 and HBO+CoQ10 groups compared to SCI group ($P < 0.05$). Furthermore, the comparison of the results between the treatment groups indicated that HBO+CoQ10 group had more volume compared to HBO and CoQ10 groups ($P < 0.05$) (Fig. 2B).

The comparison of cavity volume indicated that HBO, CoQ10 and HBO+CoQ10 groups had a lower volume in comparison with SCI group ($P < 0.05$). In addition, HBO+CoQ10 group had considerably lower cavity volume compared to HBO and CoQ10 groups ($P < 0.05$) (Fig. 2C).

Table 1
Sequence details of primers used for molecular level analysis.

Gene	Forward primer (5′ > 3′)	Reverse primer (5′ > 3′)
TNF-α	AGCCAGATCTCATACCTGCTC	GTTTGCTAGCACAGAAGCTAC
IL-1β	GACCAGCAGACGATAATCAC	TGAGTAAGACGCGGATCCAC
IL-10	GTAGCCACGCGTTGTCAGAAA	TAAGAGGGTAATGGTTCTCT
β-actin	CTAATCTATCAGCGCGCGC	TATAACGCGATGCGCAAGATC

3.1.2. The numerical densities of neuron and glial cells

Comparing the numerical density of neurons, we found that HBO, CoQ10 and HBO+CoQ10 groups were considerably higher compared to SCI group ($P < 0.05$). Moreover, HBO+CoQ10 group had significantly more neurons in comparison with HBO and CoQ10 groups ($P < 0.05$) (Fig. 2D).

Comparing the numerical density of glial cells, we observed that HBO, CoQ10 and HBO+CoQ10 groups had significantly greater density in comparison with SCI group ($P < 0.05$). Furthermore, the numerical density of glial cells in HBO+CoQ10 group in comparison with HBO and CoQ10 groups was considerably higher ($P < 0.05$) (Fig. 2E).

3.2. Combination of HBO therapy and CoQ10 administration attenuated neuronal call apoptosis after SCI

The images of immunostaining against Caspase-3 antibody are shown in Fig. 3A. In quantitative assessment of apoptotic cells, we found that HBO, CoQ10 and HBO+CoQ10 groups had significantly lower density compared to SCI group ($P < 0.05$). Furthermore, comparing of apoptotic cells density between treatment groups showed that HBO+CoQ10 group had significantly lower density in comparison with HBO and CoQ10 groups ($P < 0.05$) (Fig. 3B).

3.3. Combination of HBO therapy and CoQ10 administration modulates the oxidative status after SCI

The results of the evaluation of biochemical biomarkers are shown in Fig. 4A. The coefficients of variation (CV) for the MDA levels in the sham, SCI, HBO, CoQ10, and HBO+CoQ10 groups were estimated as 7.76%, 12.05%, 13.49%, 10.58%, and 12.63%, respectively. Comparing the MDA levels between groups indicated that HBO, CoQ10, and HBO+CoQ10 groups had considerably lower concentration in comparison with SCI group ($P < 0.05$). Moreover, the MDA concentration in HBO+CoQ10 group was considerably lower in comparison with HBO and CoQ10 groups ($P < 0.05$).

The results show that the CV in the sham, SCI, HBO, CoQ10, and HBO+CoQ10 groups for the GSH, SOD, and CAT levels were (7.08%, 13.36%, 10.48%, 10.2%, and 9.04), (5.77%, 14.36%, 12.89%, 12.25%, and 11.68%), and (8.47%, 15.01%, 13.07%, 13.33%, and 12.5%), respectively.

Comparing the GSH, SOD and CAT levels between groups, we observed that HBO, CoQ10 and HBO+CoQ10 groups compared to SCI group had significantly higher levels ($P < 0.05$). Moreover, the levels of these biomarkers were significantly higher in HBO+CoQ10 group compared to HBO and CoQ10 groups ($P < 0.05$).

3.4. Combination of HBO therapy and CoQ10 administration affected the gene expression after SCI

The results indicated that TNF-α and IL-1β genes were considerably downregulated in HBO, CoQ10 and HBO+CoQ10 groups in comparison with SCI group ($P < 0.05$). In addition, the comparison of both gene expression levels between treatment groups indicated that HBO+CoQ10 group in comparison with HBO and CoQ10 groups had significantly lower levels ($P < 0.05$) (Fig. 4B).

Furthermore, in the evaluation of IL-10 gene expression levels, the

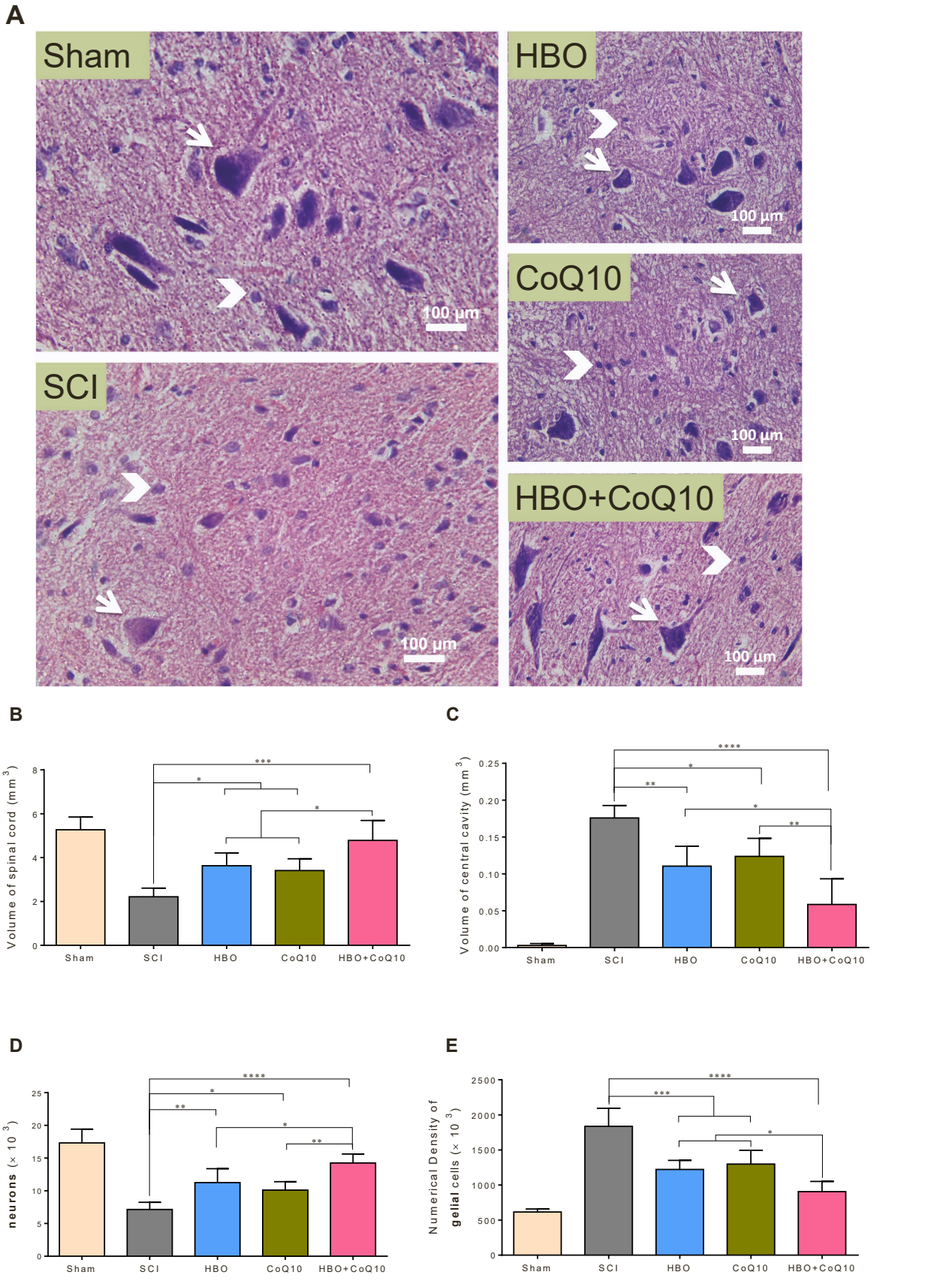

Fig. 2. The impact of HBO in combination with CoQ10 on stereological characteristics. (A) Representative micrographs of the anterior horn of spinal cord at the traumatic site stained by H&E (arrows: neuron; arrowheads: glial). Total volumes of the spinal cord (B) and central cavity (C) at the lesion site, which was determined by Cavalier's method. Numerical densities of neurons (D) and glial cells (E) at the lesion site which was determined by optical dissector method. Mean ± SD. *p < 0.05; * *p < 0.01, * **p < 0.001, * ** *p < 0.0001.

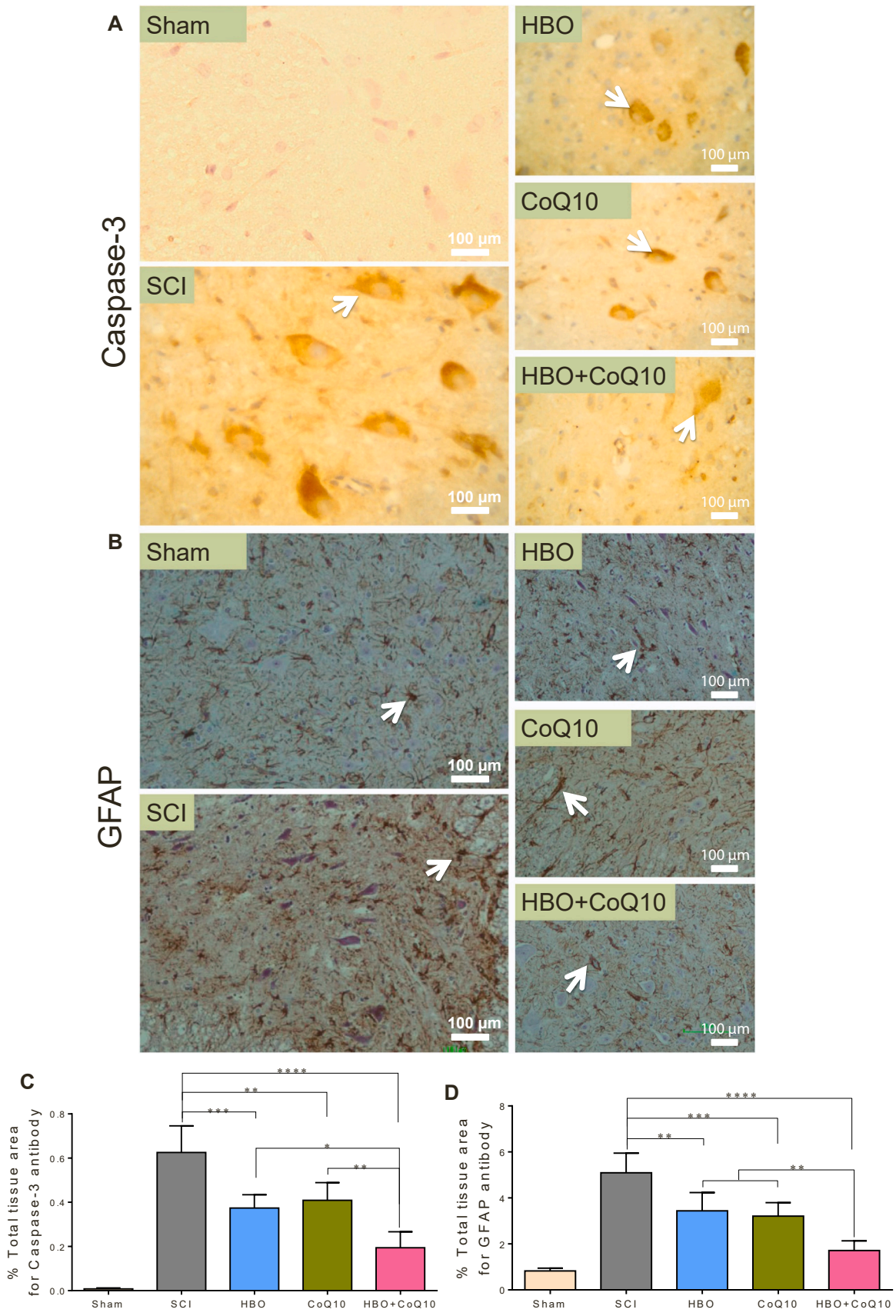

Fig. 3. The impact of HBO in combination with CoQ10 on apoptosis. (A) Immunostaining for apoptosis against Caspase-3 antibody. Arrows: positive reactions are presented in brown color. (B) Data were analyzed using densitometry. Mean ± SD. *p < 0.05; * *p < 0.01, * **p < 0.001, * ** *p < 0.0001.

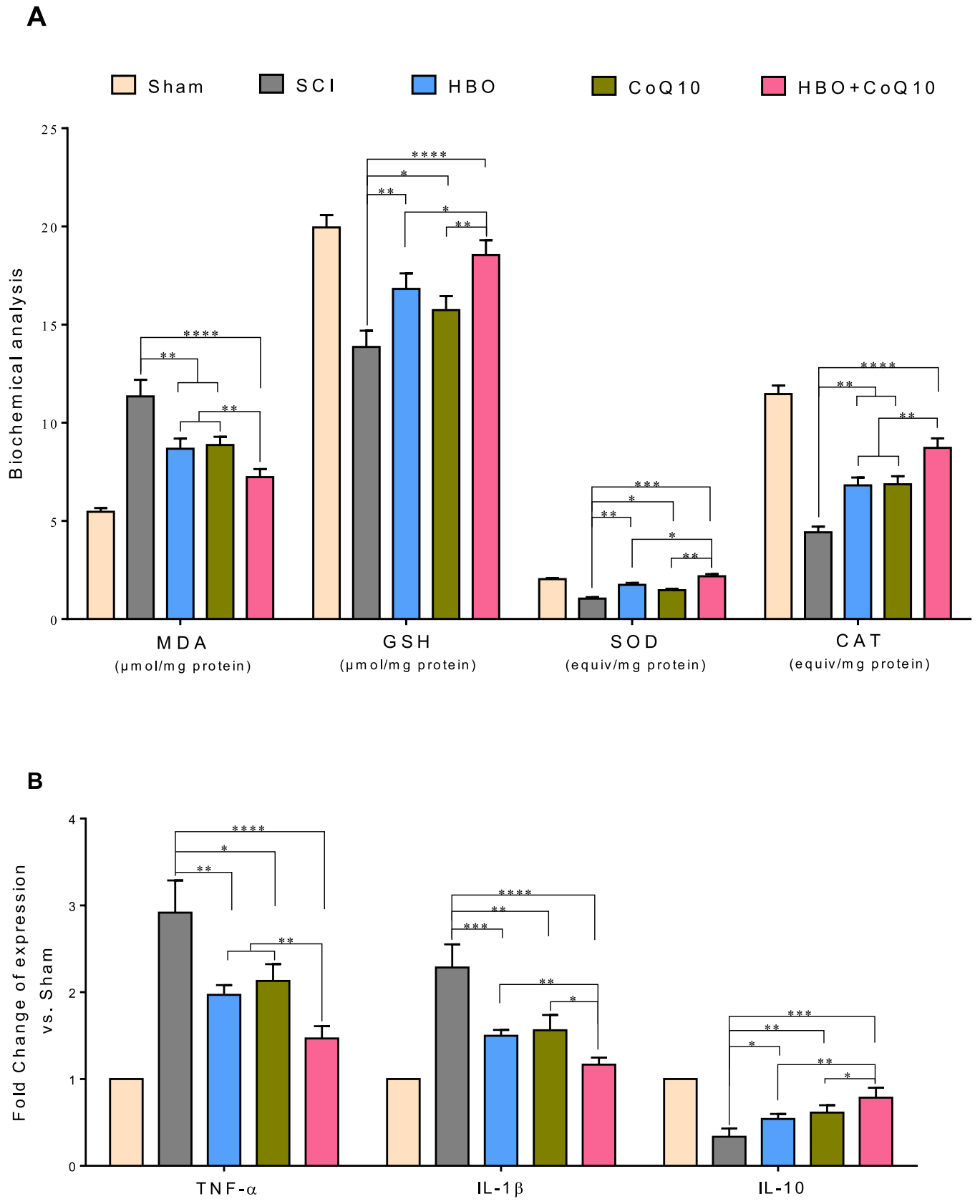

Fig. 4. The impact of HBO in combination with CoQ10 on biochemical biomarkers and gene expression levels. (A) Concentrations of oxidant (MDA) and antioxidant (GSH, SOD, and CAT) factors at the lesion site which were determined by biochemistry method. (B) Levels of immunomodulatory (IL-10) and inflammatory (TNF-α and IL-1β) genes at the lesion site which were determined by quantitative RT-PCR. Mean ± SD. *p < 0.05; * *p < 0.01, * **p < 0.001, * ** *p < 0.0001.

results showed that HBO, CoQ10 and HBO+CoQ10 groups compared to SCI group had significantly higher levels ($P < 0.05$). Also, comparisons of IL-10 gene expression levels between treatment groups, significant upregulation was observed in HBO+CoQ10 group in comparison with HBO and CoQ10 groups ($P < 0.05$) (Fig. 4B).

3.5. Combination of HBO therapy and CoQ10 administration improved neurological functions after SCI

In the current study, in order to evaluate neurological functions, three tests EMG latency, BBB and NBT were investigated. The results are shown in Fig. 5A-C.

Comparing the outcomes of the EMG latency test, the results indicated that latency of muscle response to a nerve's stimulation considerably decreased in HBO, CoQ10 and HBO+CoQ10 groups compared to SCI group ($P < 0.05$). Furthermore, HBO+CoQ10 group had significantly lower latency in comparison with CoQ10 group ($P < 0.05$).

Regarding the BBB test, we found that HBO, CoQ10 and HBO+CoQ10 groups compared to SCI group had significantly higher scores in all time points ($P < 0.05$). Furthermore, the BBB scores in HBO+CoQ10 group compared to HBO group on days 3, 7 and 14 and CoQ10 group in all time points were considerably higher ($P < 0.05$).

Regarding the NBT test, we observed that HBO, CoQ10 and HBO+CoQ10 groups had considerably higher scores in comparison with SCI group on days 1, 7 and 14 ($P < 0.05$). Moreover, comparing the results between treatment groups showed that HBO+CoQ10 group in comparison with other groups had considerably higher scores in all time points ($P < 0.05$).

4. Discussion

The time of intervention is the most important measure in preventing the spread of damage in spinal cord injuries (Mirzaie et al., 2022). Studies show that the optimal time is at the onset of damage which preventing the explosion of destructive factors such as cell apoptosis, oxidative stress and excessive inflammation at the injury site (Cheshmi et al., 2023; Mirzaie et al., 2022). Presently, we investigated whether HBO therapy and administration of CoQ10 in the early post-damage stages can attenuate the progression of injury at the lesion site of spinal cord. We observed that combination use of HBO and CoQ10 immediately after the occurrence of SCI synergistically ameliorated histological changes, reduced inflammation, apoptosis, modulated oxidative stress at the lesion site, and improved neuronal functions.

Excessive inflammation at the traumatic site of spinal cord is one of the most important destructive factors (Kwiecien et al., 2020). In this case, several pro-inflammatory cytokines such as IL-1 β and TNF-α were secreted by macrophages which invaded the site after trauma (Allison and Ditor, 2015). Studies have documented that IL-1β plays an key role in SCI, causing increases in other pro-inflammatory cytokines such as TNF-α (Burke et al., 2014; Simi et al., 2007). Therefore, inhibiting the overexpression of IL-1β can control the spread of damage and improve neurological function (Boato et al., 2013). In this regard, studies have documented that blocking the IL-1 receptor suppresses microglial activation, increases motor neuron survival, and improves nerve conduction after SCI (Schizas et al., 2014; Zong et al., 2012). Our outcomes showed a considerably downregulation of IL-1β and TNF-α genes expression levels in treatment groups, especially combined group, compared to SCI group. Moreover, we observed that the density of apoptosis, number of glial cells, and myoactivity (EMG latency) were significantly reduced in all treatment groups. This was more evident in the HBO+CoQ10 group. In addition, our study results showed significant neurological recovery (BBB and NBT scores) in the treatment groups, especially in the HBO+CoQ10 group.

In this regard, some studies have reported anti-apoptotic and anti-inflammatory effects of HBO therapy (Nasiry et al., 2022). Poyrazoglu et al. documented that HBO therapy can significantly downregulated gen expressions of IL-1β and TNF-α (Poyrazoglu et al., 2015). Furthermore, Cheshmi et al. documented that HBO therapy in early stage after SCI, reduced the expressions of TNF-α and IL-1β genes, decreased apoptosis of neurons, as well as improves motor symptoms (Cheshmi et al., 2023).

Regarding CoQ10, Al Omar et al. documented that CoQ10 has a high anti-inflammatory activity such that its administration significantly decreased TNF-α and IL-1β levels after induction of brain lead toxicity (Yousef et al., 2019). Also, they reported that administration of CoQ10 significantly downregulated the expression levels of apoptotic proteins such as Bax and Caspase-3. On the other hand, Zhang et al. reported that the administration of CoQ10 after SCI significantly reduces inflammatory mediators such as TNF-α and IL-1β and neuronal apoptosis (Zhang et al., 2015).

Due to the anti-inflammatory and anti-apoptotic effects of both HBO and CoQ10, our outcomes indicated that co-administration can more effectively in comparison with single application.

In this study, we also measured IL-10 gene expression levels. Studies have reported that the IL-10 cytokine is one of the major factors promoting neuronal survival in the spinal cord after lesion and reduces the expression of pro-inflammatory cytokines such as IF-γ, TNF-α and IL-1β (Ouyang et al., 2011; Thompson et al., 2013). We observed that IL-10 gene levels were significantly downregulated in the treatment groups, especially in HBO+CoQ10 group. Moreover, the numerical density of neurons was found to be considerably higher in the treatment group in comparison with SCI group, especially in HBO+CoQ10 group. In this regard, Cheshmi et al. demonstrated that HBO administration in spinal cord injured rats considerably upregulated the IL-10 expression levels at the lesion site, followed by a decrease in neurons apoptosis and excessive inflammation (Cheshmi et al., 2023). On the other hand, Hassanzadeh et al. reported that CoQ10 considerably upregulated IL-10 expression levels and subsequently caused a significant decrease in the Bax/Bcl2 ratio in a menopausal rat model following SCI (Hassanzadeh et al., 2018).

Oxidative stress is another major event that occurs in the early stages after trauma in the area of injury and plays a very destructive role (Raoofi et al., 2022). Oxidative stress causes the activation of apoptotic proteins and subsequent cell death. So, its control plays an important role in limiting the spread of damage (Davoodi et al., 2022; Nasiry et al., 2021).

To study the effects of treatments on biochemical status, concentration of three antioxidant factor (GSH, SOD, and CAT) and one oxidant factor, MDA, were currently measured using biochemistry method. We found that concentration of oxidant and antioxidant biomarkers were significantly decreased and increased, respectively, in all treatment groups in comparison with SCI group, and these changes were more pronounced in the HBO+CoQ10 group. Moreover, changes in these biomarkers were superior and more specific in HBO+CoQ10 group in comparison with the other two treatment groups. HBO as a nondrug and noninvasive therapy, increases the amount of dissolved oxygen in the plasma as well as saturated hemoglobin with oxygen, leading to greater oxygen availability to the organs (Michalski et al., 2011; Thom, 2011). Ahmadi et al. (2022) and Cheshmi et al. (2023) documented that levels of oxidant (MDA) and antioxidant (GSH, SOD, and CAT) factors were considerably decreased and increased, respectively, after HBO therapy in spinal cord injured animals. Furthermore, accumulating evidence indicates that in addition to improving oxygen supply and neural metabolism, there is an association between the beneficial effects of HBO therapy and a variety of biological properties, including antimicrobial effects (Memar et al., 2019), preventing reperfusion injury, strengthening immune responsiveness, encouraging new collagen (Sethuraman et al., 2022), decrease pain (Sutherland et al., 2016), accelerates neovascularization, reducing swelling, tissue perfusion, and thermoregulation in the ulcer area (Kasprzyk-Kucewicz et al., 2021).

Also, Al Omar et al. reported that treatment with CoQ10 considerably reduced the elevated level of MDA in brain tissue following lead

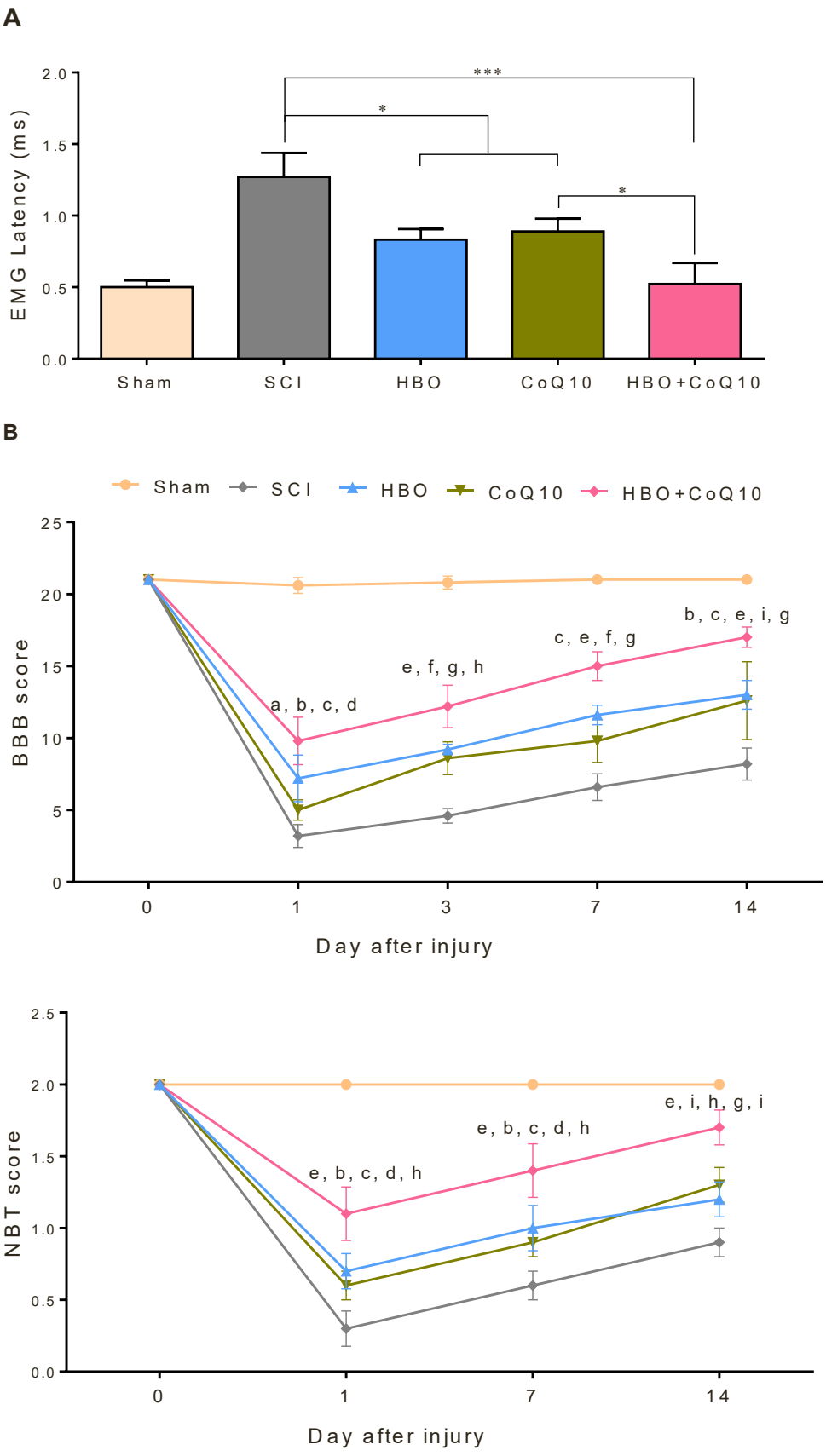

Fig. 5. The impact of HBO in combination with CoQ10 on neurological functions. (A) EMG latency test on day 14 after SCI (Millisecond; MS). (B) Basso Beattie Bresnehan (BBB) and (C) narrow beam walking tests (NBT) in difference days. Mean ± SD. *$p < 0.05$; * *$p < 0.01$, * **$p < 0.001$, * ** *$p < 0.0001$. [a] $p < 0.001$ HBO+CoQ10 group vs SCI group; [b] $p < 0.01$ HBO group vs SCI group; [c] $p < 0.05$ CoQ10 group vs SCI group; [d] $p < 0.01$ HBO+CoQ10 group vs CoQ10 group; [e] $p < 0.0001$ HBO+CoQ10 group vs SCI group; [f] $p < 0.01$ HBO+CoQ10 group vs HBO and CoQ10 groups; [g] $p < 0.001$ HBO group vs SCI group; [h] $p < 0.01$ CoQ10 group vs SCI group; [i] $p < 0.01$ HBO+CoQ10 group vs HBO group; [g] $p < 0.05$ HBO+CoQ10 group vs CoQ10 group; [h] $p < 0.05$ HBO+CoQ10 group vs HBO group; [i] $p < 0.05$ HBO group vs SCI group.

toxicity (S. Yousef et al., 2019). Furthermore, Li et al. reported that administration of CoQ10 suppresses oxidative stress through the activation of the Nrf-2/NQO-1 pathway after SCI in rats (Li et al., 2019). Therefore, considering the antioxidant effects of both compounds used in the present study, according to our results, their combined use in rats with SCI can synergistically prevent the occurrence of oxidative stress and its spread at the site of injury.

There were two limitations in the present study. First, we only conducted in vivo experiments, and the experimental data are relatively limited. Second, because female rats have been typically studied in the existing SCI literature, we chose to examine females only in this original investigation. Future investigations should examine the effects of HBO and CoQ10 in models that include males to generalize its utility.

5. Conclusion

Overall, we found that the separate administration of HBO or CoQ10 had neuroprotective effects. However, the results showed that co-administration of both compounds had synergistic effects. However, the neuroprotective effects of HBO and CoQ10 in the treatment of spinal cord injured patients require more clinical studies.

Author contributions

The study was conceptualized and designed by Alireza Ghaemi and Davood Nasiry. All authors were involved in material preparation, data collection, and analysis. The first draft of the manuscript was written by Davood Nasiry and Mohammad Darvishi and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Author statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare that all experiments protocols were approved by Ethics Committee of Mazandaran University of Medical Sciences, Sari, Iran. All methods were carried out in accordance with relevant guidelines and regulations. All authors have contributed to the writing of this article. There is no conflict of interest, and that all authors have read and approved of the manuscript being submitted.

Ethical statement

All experimental protocols were approved by Ethics Committee of Mazandaran University of Medical Sciences, Sari, Iran (Ethic no: IR.MAZUMS.4. REC.1401.14903). The animals were handled in accordant to the protocol of animal management and welfare of the University of Helsinki, Finland. Animals were kept in a laboratory standard hutch with no limitation access to a rodent's food and drinking water. All methods were carried out in accordance with relevant guidelines and regulations. The study was carried out in compliance with the ARRIVE guidelines.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Also, all authors read and approved the final manuscript.

Data Availability

Data will be made available on request.

References

Aebi, H., 1974. Catalase, Methods of Enzymatic Analysis. Elsevier, pp. 673–684.
Ahmadi, F., Zargari, M., Nasiry, D., Khalatbary, A.R., 2022. Synergistic neuroprotective effects of hyperbaric oxygen and methylprednisolone following contusive spinal cord injury in rat. J. Spinal Cord. Med 45, 930–939.
Alizadeh, A., Dyck, S.M., Karimi-Abdolrezaee, S., 2019. Traumatic spinal cord injury: an overview of pathophysiology, models and acute injury mechanisms. Front Neurol. 10, 282.
Allison, D., Ditor, D., 2015. Immune dysfunction and chronic inflammation following spinal cord injury. Spinal Cord. 53, 14–18.
Anjum, A., Yazid, M.Di, Fauzi Daud, M., Idris, J., Ng, A.M.H., Selvi Naicker, A., Ismail, O. H.R., Athi Kumar, R.K., Lokanathan, Y., 2020. Spinal cord injury: pathophysiology, multimolecular interactions, and underlying recovery mechanisms. Int J. Mol. Sci. 21, 7533.
Armand, C., Grzegorz, K., Agata, S., Marek, K., Mariusz, N., Aleksander, S., Zofia, D., 2012. Thermal imaging and TC oximetry measurements of hyperbaric oxygen therapy (HBO) effects on trophic ulceration of the crura. J. Therm. Anal. Calor. 108, 25–31.
Basso, D.M., Beattie, M.S., Bresnahan, J.C., 1996. Graded histological and locomotor outcomes after spinal cord contusion using the NYU weight-drop device versus transection. Exp. Neurol. 139, 244–256.
Boato, F., Rosenberger, K., Nelissen, S., Geboes, L., Peters, E.M., Nitsch, R., Hendrix, S., 2013. Absence of IL-1β positively affects neurological outcome, lesion development and axonal plasticity after spinal cord injury. J. Neuroinflamm. 10, 1–11.
Burke, S.J., Lu, D., Sparer, T.E., Karlstad, M.D., Collier, J.J., 2014. Transcription of the gene encoding TNF-α is increased by IL-1β in rat and human islets and β-cell lines. Mol. Immunol. 62, 54–62.
Cheshmi, H., Mohammadi, H., Akbari, M., Nasiry, D., Rezapour-Nasrabad, R., Bagheri, M., Abouhamzeh, B., Poorhassan, M., Mirhoseini, M., Mokhtari, H., 2023. Human placental mesenchymal stem cell-derived exosomes in combination with hyperbaric oxygen synergistically promote recovery after spinal cord injury in rats. Neurotox. Res 1–15.
Christie, S.D., Comeau, B., Myers, T., Sadi, D., Purdy, M., Mendez, I., 2008. Duration of lipid peroxidation after acute spinal cord injury in rats and the effect of methylprednisolone. Neurosurg. Focus 25, E5.
Citron, B.A., Arnold, P.M., Sebastian, C., Qin, F., Malladi, S., Ameenuddin, S., Landis, M. E., Festoff, B.W., 2000. Rapid upregulation of caspase-3 in rat spinal cord after injury: mRNA, protein, and cellular localization correlates with apoptotic cell death. Exp. Neurol. 166, 213–226.
David, G., Mohammadi, S., Martin, A.R., Cohen-Adad, J., Weiskopf, N., Thompson, A., Freund, P., 2019. Traumatic and nontraumatic spinal cord injury: pathological insights from neuroimaging. Nat. Rev. Neurol. 15, 718–731.
Davoodi, S., Ebrahimpour-Malekshah, R., Ayna, Ö., Akbari, M., Raoofi, A., Mokhtari, H., Izanlu, M., Modanloo, F., Nasiry, D., 2022. Decellularized human amniotic membrane engraftment in combination with adipose-derived stem cells transplantation, synergistically improved diabetic wound healing. J. Cosmet. Dermatol. 21, 6939–6950.
Ellman, G., 1959. Tissue sulfhydryl groups. Arch. Biochem Biophys. 82, 70–77.
Hassanzadeh, S., Jameie, S.B., Soleimani, M., Farhadi, M., Kerdari, M., Danaei, N., 2018. Coenzyme Q10 influences on the levels of TNF-α and IL-10 and the ratio of Bax/Bcl2 in a menopausal rat model following lumbar spinal cord injury. J. Mol. Neurosci. 65, 255–264.
Howard, V., Reed, M., 2004. Unbiased Stereology: Three-dimensional Measurement in Microscopy. Garland Science.
Huang, L., Zhang, Q., Fu, C., Liang, Z., Xiong, F., He, C., Wei, Q., 2021. Effects of hyperbaric oxygen therapy on patients with spinal cord injury: a systematic review and meta-analysis of randomized controlled trials. J. Back Musculoskelet. Rehabil. 34, 905–913.
Izanlu, M., Khalatbary, A., Aliabadi, A., Davoodi, S., Raoofi, A., Modanloo, F., Nasiry, D., 2022. Synergistic effect of hyperbaric oxygen and decellularized human amniotic membrane on full-thickness diabetic wound healing in rats. J. Maz. Univ. Med Sci. 32, 1–15.
Karsy, M., Hawryluk, G., 2019. Modern medical management of spinal cord injury. Curr. Neurol. Neurosci. Rep. 19, 1–7.
Kasprzyk-Kucewicz, T., Cholewka, A., Englisz-Jurgielewicz, B., Mucha, R., Relich, M., Kawecki, M., Sieroń, K., Onak, P., Stanek, A., 2021. Thermal effects of topical hyperbaric oxygen therapy in hard-to-heal wounds—a pilot study. Int J. Environ. Res Public Health 18, 6737.
Keller, A.V., Rees, K.M., Seibt, E.J., Wood, B.D., Wade, A.D., Morehouse, J., Shum-Siu, A., Magnuson, D.S., 2018. Electromyographic patterns of the rat hindlimb in response to muscle stretch after spinal cord injury. Spinal Cord. 56, 560–568.
Kirby, J.P., Snyder, J., Schuerer, D.J., Peters, J.S., Bochicchio, G.V., 2019. Essentials of hyperbaric oxygen therapy: 2019 review. Mo Med 116, 176.
Kwiecien, J.M., Dabrowski, W., Dąbrowska-Bouta, B., Sulkowski, G., Oakden, W., Kwiecien-Delaney, C.J., Yaron, J.R., Zhang, L., Schutz, L., Marzec-Kotarska, B., 2020. Prolonged inflammation leads to ongoing damage after spinal cord injury. PLoS One 15, e0226584.
Lalieu, R., Raap, R.B., van Hulst, R., 2022. Hyperbaric oxygen therapy: when pressure is good for diabetic foot ulcers. Br. J. Community Nurs. 27, S6–S12.

Li, X., Zhan, J., Hou, Y., Chen, S., Hou, Y., Xiao, Z., Luo, D., Lin, D., 2019. Coenzyme Q10 suppresses oxidative stress and apoptosis via activating the Nrf-2/NQO-1 and NF-κB signaling pathway after spinal cord injury in rats. Am. J. Transl. Res 11, 6544.
Liu, Z., Yang, Y., He, L., Pang, M., Luo, C., Liu, B., Rong, L., 2019. High-dose methylprednisolone for acute traumatic spinal cord injury: a meta-analysis. Neurology 93, e841–e850.
Lu, C.-J., Guo, Y.-Z., Zhang, Y., Yang, L., Chang, Y., Zhang, J.-W., Jing, L., Zhang, J.-Z., 2017. Coenzyme Q10 ameliorates cerebral ischemia reperfusion injury in hyperglycemic rats. Pathol. Res Pr. 213, 1191–1199.
Madmani, M.E., Solaiman, A.Y., Agha, K.T., Madmani, Y., Shahrour, Y., Essali, A., Kadro, W., 2014. Coenzyme Q10 for heart failure. Cochrane Database Syst. Rev.
Memar, M.Y., Yekani, M., Alizadeh, N., Baghi, H.B., 2019. Hyperbaric oxygen therapy: antimicrobial mechanisms and clinical application for infections. Biomed. Pharm. 109, 440–447.
Michalski, D., Härtig, W., Schneider, D., Hobohm, C., 2011. Use of normobaric and hyperbaric oxygen in acute focal cerebral ischemia–a preclinical and clinical review. Acta Neurol. Scand. 123, 85–97.
Mirzaie, J., Nasiry, D., Ayna, Ö., Raoofi, A., Delbari, A., Rustamzadeh, A., Nezhadi, A., Jamalpoor, Z., 2022. Neuroprotective effects of lovastatin against traumatic spinal cord injury in rats. J. Chem. Neuroanat. 125, 102148.
Misra, H.P., Fridovich, I., 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247, 3170–3175.
Nasiry, D., Khalatbary, A.R., Abdollahifar, M.-A., Bayat, M., Amini, A., Ashtiani, M.K., Rajabi, S., Noori, A., Piryaei, A., 2022. SDF-1α loaded bioengineered human amniotic membrane-derived scaffold transplantation in combination with hyperbaric oxygen improved diabetic wound healing. J. Biosci. Bioeng. 133, 489–501.
Nasiry, D., Khalatbary, A.R., Ahmadvand, H., Talebpour Amiri, F.B., 2021. Effects of Juglans regia L. leaf extract supplementation on testicular functions in diabetic rats. Biotech. Histochem 96, 41–47.
Ouyang, W., Rutz, S., Crellin, N.K., Valdez, P.A., Hymowitz, S.G., 2011. Regulation and functions of the IL-10 family of cytokines in inflammation and disease. Annu Rev. Immunol. 29, 71–109.
Poyrazoglu, Y., Topal, T., Yuksel, R., Bircan, F.S., Simsek, K., Gocgeldi, E., Ersoz, N., Korkmaz, A., 2015. Effects of hyperbaric oxygen and preconditioning on wound healing in colonic anastomoses. J. Invest Surg. 28, 188–195.
Raizner, A.E., 2019. Coenzyme Q10. Methodist Debakey Cardiovasc J. 15, 185.
Raoofi, A., Delbari, A., Nasiry, D., Eslampour, H., Golmohammadi, R., Sadat Javadinia, S., Sadrzadeh, R., Mojadadi, M.-S., Rustamzadeh, A., Akhlaghi, M., 2022. Caffeine modulates apoptosis, oxidative stress, and inflammation damage induced by tramadol in cerebellum of male rats. J. Chem. Neuroanat. 123, 102116.
Yousef, S., A.O, Fahad, A, A., Abdel Moneim, A.E., Metwally, D.M., El-Khadragy, M.F., Kassab, R.B., 2019. The neuroprotective role of coenzyme Q10 against lead acetate-induced neurotoxicity is mediated by antioxidant, anti-inflammatory and anti-apoptotic activities. Int J. Environ. Res Public Health 16, 2895.
Saha, R., Roychoudhury, S., Kar, K., Varghese, A., Nandi, P., Sharma, G., Formicki, G., Slama, P., Kolesarova, A., 2019. Coenzyme Q10 ameliorates cadmium induced reproductive toxicity in male rats. Physiol. Res 68, 141–145.
Samimi, F., Baazm, M., Eftekhar, E., Jalali Mashayekh, F., 2019. Effect of coenzyme Q10 supplementation on liver total oxidant/antioxidant status in streptozotocin-induced diabetic rats. J. Arak Univ. Med. Sci. 22, 28–39.
Schizas, N., Andersson, B., Hilborn, J., Hailer, N.P., 2014. Interleukin-1 receptor antagonist promotes survival of ventral horn neurons and suppresses microglial activation in mouse spinal cord slice cultures. J. Neurosci. Res 92, 1457–1465.
Sethuraman, K.N., Smolin, R., Henry, S., 2022. Is there a place for hyperbaric oxygen therapy? Adv. Surg. 56, 169–204.
Simi, A., Tsakiri, N., Wang, P., Rothwell, N., 2007. Interleukin-1 and inflammatory neurodegeneration. Biochem Soc. Trans. 35, 1122–1126.
Sun, L., Zhao, L., Li, P., Liu, X., Liang, F., Jiang, Y., Kang, N., Gao, C., Yang, J., 2019. Effect of hyperbaric oxygen therapy on HMGB1/NF-κB expression and prognosis of acute spinal cord injury: a randomized clinical trial. Neurosci. Lett. 692, 47–52.
Sutherland, A.M., Clarke, H.A., Katz, J., Katznelson, R., 2016. Hyperbaric oxygen therapy: a new treatment for chronic pain? Pain. Pr. 16, 620–628.
Thom, S.R., 2011. Hyperbaric oxygen: its mechanisms and efficacy. Plast. Reconstr. Surg. 127, 131S–141S.
Thompson, C.D., Zurko, J.C., Hanna, B.F., Hellenbrand, D.J., Hanna, A., 2013. The therapeutic role of interleukin-10 after spinal cord injury. J. Neurotrauma 30, 1311–1324.
Uchiyama, M., Mihara, M., 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal. Biochem 86, 271–278.
von Euler, M., Åkesson, E., Samuelsson, E.-B., Seiger, Å., Sundström, E., 1996. Motor performance score: a new algorithm for accurate behavioral testing of spinal cord injury in rats. Exp. Neurol. 137, 242–254.
Zhang, X.X., Qian, K.J., Zhang, Y., Wang, Z.J., Yu, Y.B., Liu, X.J., Cao, X.T., Liao, Y.H., Zhang, D.Y., 2015. Efficacy of coenzyme Q10 in mitigating spinal cord injury-induced osteoporosis. Mol. Med Rep. 12, 3909–3915.
Zong, S., Zeng, G., Wei, B., Xiong, C., Zhao, Y., 2012. Beneficial effect of interleukin-1 receptor antagonist protein on spinal cord injury recovery in the rat. Inflammation 35, 520–526.