ELSEVIER

Archives of Medical Research 41 (2010) 506–512

Archives of Medical Research

ORIGINAL ARTICLE

Combined Hyperbaric Oxygen and Hypothermia Treatment on Oxidative Stress Parameters after Spinal Cord Injury: An Experimental Study

Kivanc Topuz,[a] Ahmet Colak,[a] Berker Cemil,[e] Murat Kutlay,[a] Mehmet Nusret Demircan,[a] Hakan Simsek,[a] Osman Ipcioglu,[b] Zafer Kucukodaci,[c] and Gunalp Uzun[d]

[a]*Department of Neurosurgery,* [b]*Department of Biochemistry,* [c]*Department of Pathology,*
[d]*Department of Underwater and Hyperbaric Medicine, Haydarpasa Training Hospital, Gulhane Military Medical Academy, Istanbul, Turkey*
[e]*Department of Neurosurgery, Fatih University, Faculty of Medicine, Ankara, Turkey*

Received for publication June 27, 2010; accepted September 30, 2010 (ARCMED-D-10-00330).

Background and Aims. We undertook this study to investigate the possible beneficial effects of combined hypothermia and hyperbaric oxygen (HBO) treatment in comparison with methylprednisolone in experimental spinal cord injury (SCI).

Methods. Forty eight male Wistar albino rats (200–250 g) were randomized into six groups; A (normothermic control group; only laminectomy), B (normothermic trauma group; laminectomy + spinal trauma), C (normothermic methylprednisolone group; laminectomy + spinal trauma + methylprednisolone treated), D (hypothermia group; laminectomy + spinal trauma + hypothermia treated); E (HBO group; laminectomy + spinal trauma + HBO therapy), F (hypothermia and HBO group; laminectomy + spinal trauma + hypothermia and HBO treated) each containing eight rats. Neurological assessments were performed 24 h after trauma and spinal cord tissue samples had been harvested for both biochemical and histopathological evaluation.

Results. After SCI, tissue malondialdehyde (MDA) level of the control group was measured increased, and superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) enzyme activities were measured decreased. In group F, it was also shown that MDA level elevation had been prevented, and group F has increased the antioxidant enzyme activities than the other experimental groups C, D, E ($p < 0.05$).

Conclusions. We concluded that the use of combined hypothermia and HBO treatment might have potential benefits in spinal cord tissue on secondary damage.

Key Words: Hyperbaric oxygen, Hypothermia, Lipid peroxidation, Anti-oxidant enzymes, Spinal cord injury.

Introduction

Traumatic spinal cord injury (SCI) includes primary and secondary injury mechanisms (1). The mechanical effects of the trauma are the primary cause of damage to the spinal cord (2). Primary injury is immediate and irreversible and results in direct damage to neuronal and vascular tissues. Secondary injury, due to disruption of cell membranes and alterations in both spinal cord blood flow and metabolism, exacerbates the effects of initial mechanical trauma (1–4). The majority of experimental studies were designed to clarify the underlying physiopathological mechanism of secondary injury (5). Spinal cord microvascular potency and blood flow decrease (causing ischemia) just after severe contusion or compression injury (1,2). Many experimental studies suggested that formation of oxygen radicals and cell membrane lipid peroxidation play important roles in pathogenesis of secondary damage (5–8).

In accordance with the recent advances in SCI investigations, many treatment regimens such as receptor blockers, physiological antagonists, inhibitors of biosynthetic pathways and membrane-stabilizing drugs have come into use

Address reprint requests to: Berker Cemil, Department of Neurosurgery, Fatih University Faculty of Medicine, 06510, Ankara, Turkey; Phone: +90 312 503 5131; E-mail: berker5@yahoo.com

0188-4409/$ - see front matter.
doi: 10.1016/j.arcmed.2010.10.004

over time. Still, there is no effective treatment to remedy the detrimental effects of SCI (9). The application of hypothermia by lowering body or spinal cord temperature after SCI has been used successfully as a neuroprotective adjunct for spinal cord trauma in rodents (10). The protective effects of hypothermia are attributed to suppression of the injury-induced immune response and inflammation (10). Hypothermia also retards excitotoxicity and inhibits oxidative stress through a reduction in oxygen free radical production and by promoting an increase in spinal collateral blood flow (10). In experimental models, several studies present the effect of HBO on SCI (11,12), but the underlying mechanism is poorly understood. This therapy results in an increase in tissue oxygen tension and improves collagen synthesis, angiogenesis, epithelization, and resistance to bacteria in problem wounds (13,14).

Hypothermia and HBO treatments for SCI have been well described in the literature (2,15). To date, according to our literature search, no combination therapy of hypothermia and HBO has been reported. The aim of this study was to determine the effect of a combination of hypothermia and HBO therapy and compare with methylprednisolone (MP) treatment on oxidative status after experimental SCI by measuring lipid peroxidation (MDA) and antioxidant enzymes, namely, SOD, GSH-Px, CAT, and MDA.

Materials and Methods

Animals and Study Groups

This study was performed using 48 male Wistar albino rats weighing 200−250 g. Animal protocols were approved by the Ethics Committee for Care and Use of Laboratory Animals of Marmara University. Rats were housed in individual cages in the animal laboratory 1 month prior to the initiation of the experiment. They were allowed free access to food and water ad libitum before and after surgery and were maintained in a 12 h light/dark cycle at room temperature (22 ± 3°C). Each rat was tested and normal motor function was found before surgery.

Rats were randomly divided into six experimental groups: A (normothermic control group; only laminectomy), B (normothermic trauma group; laminectomy + spinal trauma), C (normothermic MP group; laminectomy + spinal trauma + MP treated), D (hypothermia group; laminectomy + spinal trauma + hypothermia treated); E (HBO group; laminectomy + spinal trauma + HBO therapy), F (hypothermia and HBO group; laminectomy + spinal trauma + hypothermia treated + HBO treated), each of which contained eight rats.

Surgical Technique

All rats were anesthetized with an intramuscular injection of ketamine hydrochloride (50 mg/kg; Ketalar, Parke-Devis, Istanbul, Turkey) plus xylazine hydrochloride (10 mg/kg; Rompun, Bayer, Istanbul, Turkey). Rats were positioned prone on the operating table. During surgery, except for the animals treated with hypothermia, the rats were kept in a heating fan connected to a box to maintain body temperature at 37°C as measured by rectal probe. A midline dorsal incision using sterile technique was performed. T8-L2 laminae were exposed by dissection of paravertebral muscles. Laminectomy was performed at T9-L1 levels. SCIs as described by Rivlin and Tator (16) were accomplished by extradural compression of the exposed spinal cords at T10-12 level for 60 sec using an aneurysm clip (Yasargil FE 760, force of closure 70 g) in every case. The following paravertebral fascia and skin were sutured.

Treatments and Study Design

In group A, laminectomy was performed but clip was not applied. In group B, no medication was given after clip application. In group C, all rats received an IP injection of MP (30 mg/kg; Prednol-L, Mustafa-Nevzat, Istanbul, Turkey) after clip application. In group D, the injured spinal cord segments were treated with hypothermia for 120 min after clip application. The animals reached the target temperature 27−29°C within 30 min following hypothermic treatment application. In group E, a steel animal hyperbaric oxygen chamber was flushed with 100% oxygen at the beginning. The chamber pressure was increased to 2.4 atmospheres absolute (ATA) in 10 min and decompression to normobaric air at the end of the session gradually in 5 min. HBO session was set as 90 min and was administered following clip application. In group F, hypothermia treatment was applied in the hyperbaric oxygen chamber for 90 min. Hyperbaric treatment was started after the animals reached the target temperature 27−29°C within 30 min following hypothermic treatment application.

Control of Rat Body Temperature

To control and maintain the systemic temperature of the rats, a heating fan connected to a box (60 x 30 x 30 cm) in which the animals were placed was used as described previously (17). The rats' rectal temperature was continuously monitored with precision thermometer Ellab DM 852. Following SCI, the rats were placed in the box, and their body temperatures were maintained for 120 min under hypothermic (27−29°C) conditions. Animals reached the target temperature for hypothermia within 30 min of application. After treatment, rats were returned to their cages and body temperature was allowed to recover naturally.

Neurological Evaluation

Neurological signs after SCI were examined with the inclined plane test as described by Rivlin and Tator (16). In the inclined plane test, rats were placed on a flat platform. The angle of the inclined plane is the maximum angle of the platform at which an animal can support its weight

on an inclined plane measured at 0–90°C on which the rat could keep itself in position at least for 5 min.

Sample Collection

Twenty four hours after clip application the rats were anesthetized again and then were sacrificed. Spinal cord segments were excised for a length of 5 mm rostrally and caudally to the injury site. All removed samples were thoroughly cleaned of blood with a scalpel and each sample was allocated in two parts. Rostral part was immediately frozen and stored at −20°C for biochemical analyses and the caudal part was fixed and stored at 4°C for histopathological evaluation.

Biochemical Analysis

All removed traumatized tissue samples were homogenized (for 2 min at 16,000 rpm) in 1 mL phosphate-buffered saline using a homogenizer (T25 Janke & Kunkel GMBH, IKA-Labortechnik, Staufen, Germany) after cutting the samples into small pieces. The supernatant solution was collected and analyzed for enzyme activity and protein concentration. Protein assays in the samples were measured in the homogenate according to the method of Lowry et al. (18). All procedures were carried out at +4°C.

Quantitative measurement of lipid peroxidation as MDA was performed using a Malondialdehyde Assay Kit (NWK-MDA01, Northwest Life Science Specialties LLC, Vancouver, WA). Results were expressed as micromoles of MDA per gram tissue (μmol/g protein). The supernatant solution was evaluated for SOD activity using a Superoxide Dismutase Assay kit (#706002; Cayman Chemicals Inc., Ann Arbor, MI) according to the manufacturer's protocol. SOD activity was expressed as units per gram protein (U/g protein). The CAT assay kit was obtained from Cayman Chemicals (cat. #707002) and the assays were conducted according to their instructions for evaluating CAT activity. Activity was given in micromoles per gram protein (μmol/g protein). To measure the GSH-Px activity, the GSH-Px assay kit was used (test kit code no. NWK-GPX01, Northwest Life Science Specialties LLC) by adapting the method of Paglia and Valentine (19). Activity was given in milliunits per gram protein (mU/g protein).

Pathological Assessment

The pathological samples were fixed in 10% neutral buffered formalin and stored at 4°C for 1 week. The spinal cords were embedded in paraffin. Each block was sectioned coronally at 5 μm and trimmed with Leica microtome (Leica RM2035, Germany). Sections were stained with hematoxylin and eosin (H&E). Preparations were evaluated by a brightfield microscope (Olympus BH-2, Tokyo, Japan) and were photographed (Sony CCD-IRIS, Sony Inc, Tokyo, Japan). All sections were evaluated by the same pathologist who was blinded to the treatment groups for presence of hemorrhage, cellular edema, necrosis, and neutrophil infiltration.

Statistical Analysis

All data were carried out using SPSS program for statistical calculations (Statistical Package for Social Sciences for Windows, v.15.0). The parameters were analyzed with Kruskal-Wallis test to determine differences between groups, and Mann-Whitney U test was used for dual comparison; p value <0.05 was considered statistically significant.

Results

Neurological Findings

The control group showed statistically significant results compared to the other groups. When all treatment groups (groups C–F) had been compared, statistically difference was not found among these groups ($p > 0.05$). However, group F showed better results than the other treatment groups. The results of neurological findings are summarized in Figure 1.

Biochemical Findings

Results of MDA, SOD, CAT, and GSH-Px are summarized in Figure 2. The highest MDA level was found in group B when compared with all other groups. In addition, MDA levels in the spinal cord tissues were found to be significantly lower in groups C–F than in group B. However, MDA levels were not significantly different among groups B, C, and D ($p > 0.05$). Groups E and F were not

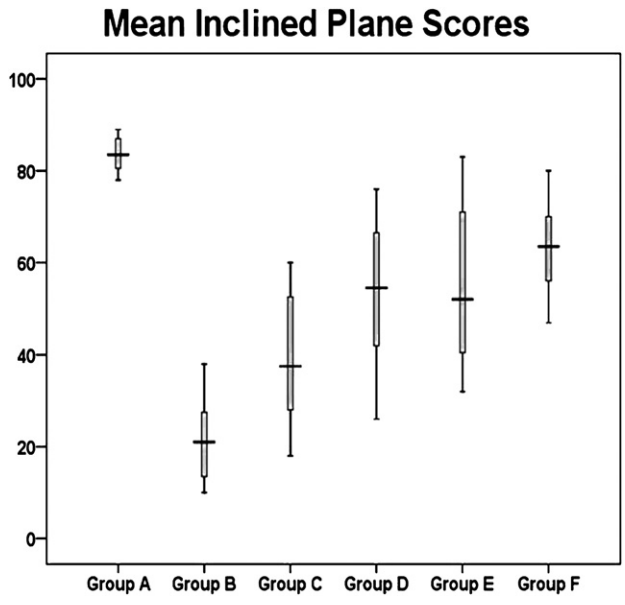

Figure 1. Box-and-whisker plot showing inclined plane scores of rat groups.

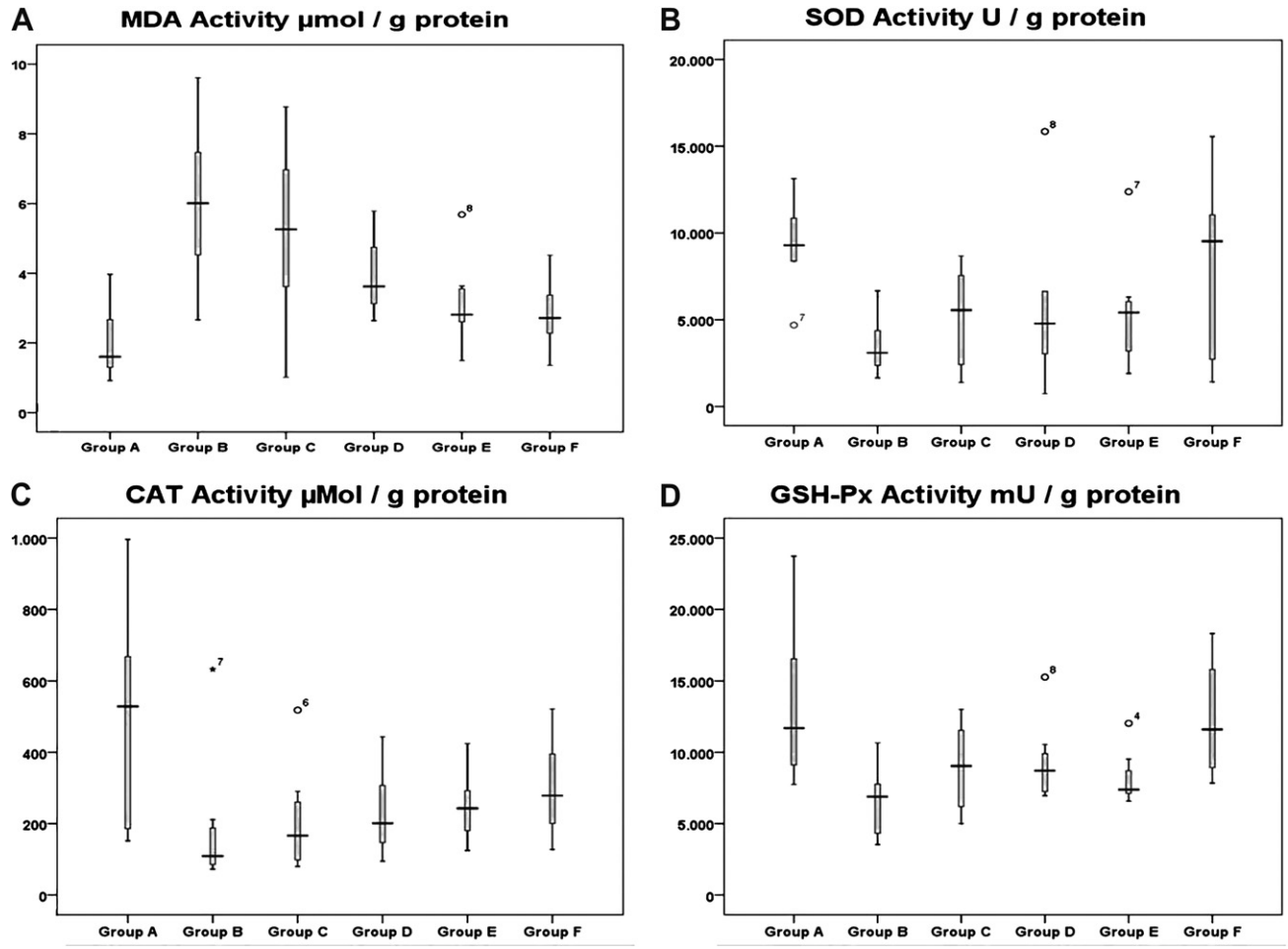

Figure 2. Box-and-whisker plot showing: (A) MDA activities in the spinal cord tissues. MDA level was found highest in group B when compared with all other groups. (B) Group F revealed significantly increased SOD levels in groups C−E ($p < 0.05$). (C) CAT activities in the spinal cord tissues; group F showed significantly increased CAT levels compared to groups C−E ($p < 0.05$). (D) GSH-Px activities in the spinal cord tissues. Group F had increased GSH-Px levels compared to the other treatment groups ($p < 0.05$). Any outliers are marked with a circle and extreme cases with an asterisk. They are marked by their row number.

statistically different ($p > 0.05$) when compared to group A ($p > 0.05$) (Figure 2A).

SOD levels were lower in group B compared to the other groups. A statistically significant difference was not found between SOD levels in group A and group F. Group F revealed significantly increased SOD levels in groups C−E ($p < 0.05$) (Figure 2B).

CAT levels were not significantly different between group A and groups D−F ($p > 0.05$). In addition, a statistical difference was not shown between CAT levels in group C and group B ($p > 0.05$). Group F showed significantly increased CAT levels compared to groups C−E ($p < 0.05$) (Figure 2C).

GSH-Px levels were significantly different between groups B and E ($p < 0.05$). On the other hand, GSH-Px levels in groups B and E were significantly different from group A ($p < 0.05$). Group F had increased GSH-Px levels compared to the other treatment groups ($p < 0.05$) (Figure 2D).

Briefly; in group B, activities of the antioxidant enzymes (SOD, CAT, and GSH-Px) were found to be significantly lower than in the groups A, C, D, E, and F. In groups C−F, there was a considerable increase in activities of these enzymes in the spinal cord tissues when compared to group B.

Pathological Findings

Group A demonstrated normal pathological structure (Figure 3). In group B, animals had hemorrhage, cellular edema, necrosis, neutrophil infiltration, and total destruction of cells with pronounced vacuolization (Figure 4). In groups C−E; the pathological findings were almost similar. Many animals had an intermediate degree of hemorrhage, cellular edema, necrosis, cell destruction with hyperchromatic nuclei, and adjacent normal structured cells (Figure 5). In group F sections, evidences of hemorrhage, cellular edema, and neutrophil infiltration were also found reduced from the others (Figure 6).

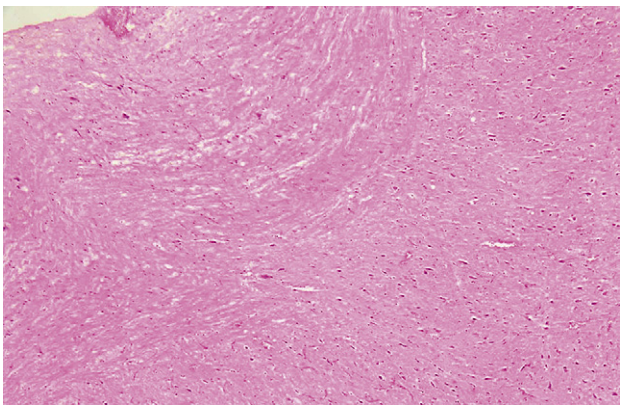

Figure 3. Spinal cord pathological section in group A [hematoxylin and eosin, (H&E), x40]. (A color figure can be found in the online version of this article.)

Discussion

Traumatic SCIs are primarily caused by mechanical forces. Secondary injury is considered to be the result of a number of proposed self-destructive processes. Both acute inflammatory response at the site of injury and spinal cord hemorrhage with release of Fe and hemoproteins yield the production of reactive oxygen species (ROS) and cytotoxic edema which, in turn, contribute to lipid peroxidation and ischemia (20–25). The central nervous system (CNS) is particularly vulnerable to free-radical oxidation following hypoxic or traumatic injuries because of their high unsaturated lipid content (21). Although there is no effective medical or surgical treatment for primary damage, it seems theoretically possible to uncover the mechanisms of secondary changes and prevent them from taking place.

Numerous investigators have postulated that free radical-triggered peroxidative events occur after SCI.

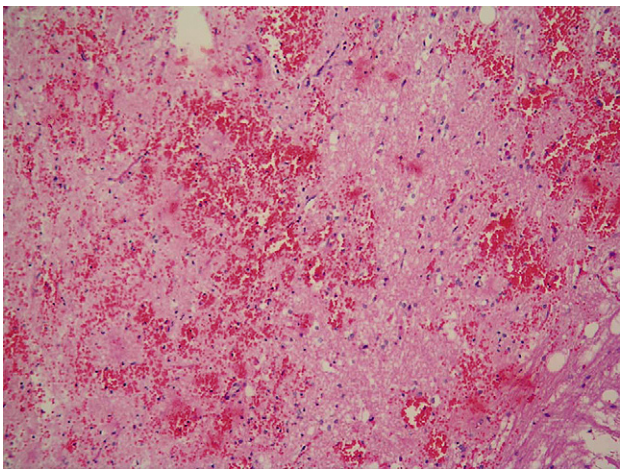

Figure 4. Spinal cord pathological section in group B; laminectomy + spinal trauma (H&E x100). (A color figure can be found in the online version of this article.)

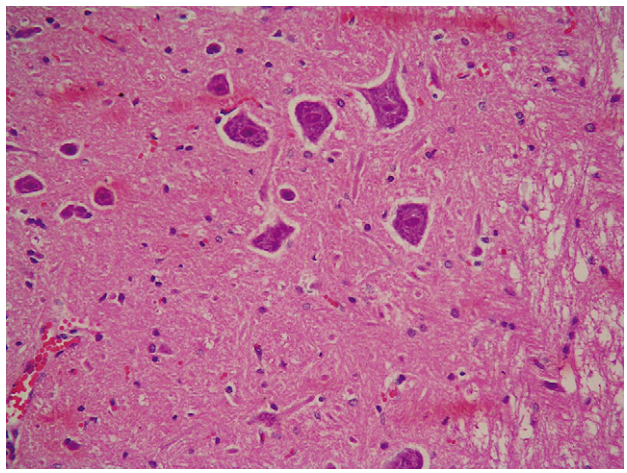

Figure 5. Spinal cord pathological section in group C; laminectomy + spinal trauma + methylprednisolone treated (H&E x200). (A color figure can be found in the online version of this article.)

Therefore, it is important to prevent lipid peroxidation for neurological recovery. Neuroprotective effect of high dose MP on SCI has been well described previously (26). It suppresses posttraumatic lipid peroxidation and free radical generation and hydrolysis at the site of injury (1,26). SCI significantly increased spinal cord tissue malondialdehyde (MDA) and also decreased superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) enzyme activities compared to control (5). Serarslan et al. reported that MP treatment decreases tissue MDA levels and prevents inhibition of the enzymes SOD and GSH-Px in tissues (1). In the present study, in group B increased levels of MDA and decreased levels of SOD, GSH-Px,

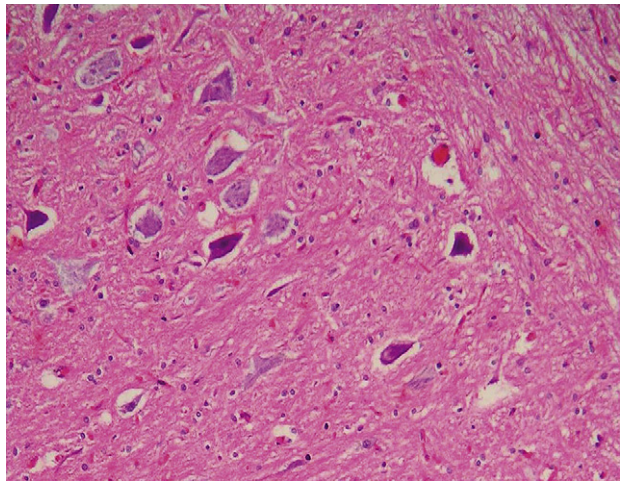

Figure 6. Spinal cord pathological section in group F; laminectomy + spinal trauma + hypothermia and HBO treated (H&E x200). Less degenerative changes and slight shrinking in cytoplasm and nucleus are seen in neuronal cells. (A color figure can be found in the online version of this article.)

and CAT were found. Our results are compatible with the previous reports. In addition, increased levels of SOD, GSH-Px, and CAT and decreased levels of MDA were also found in group C. These findings show concordance with the results of Serarslan et al. (1).

HBO is a very interesting therapeutic modality, which is known to reduce oxidative stress itself when used in pathological conditions (27,28). HBO therapy has been shown, in experimental models, to ameliorate the hypoxic state induced by edema and circulatory compromise after SCI (11,29,30). In addition, HBO treatment is neuroprotective in cerebral ischemia and brain trauma (31−34). HBO decreases edema and promotes capillary angiogenesis (13,35). The biological effect of hyperbaric oxygen is derived from hypersaturating circulating plasma with dissolved oxygen during and shortly after treatment, resulting in a transiently increased diffusion gradient between the circulation and surrounding tissues driving transport of oxygen into the interstitium and tissues (13,35,36). Previously, Aydinoz et al. reported that the beneficial effect of HBO on cisplatin-induced nephrotoxicity seems to be partially mediated by a significant reduction in lipid peroxidation and by an increase in SOD and GSH-Px activities in the kidneys. In our study, HBO-treated groups showed that HBO decreased MDA levels and increased SOD, GSH-Px, and CAT levels. These findings are in accordance with the results of Aydinoz et al. Our data on pathological examination indicate that administration of HBO attenuated traumatic injury. In addition, the results of neurological behavioral outcome of HBO-treated groups were consistent.

Hypothermia was found to have a protective effect which is complex and influenced by several different biochemical cascades during SCI (2). Hypothermia contributes to neuroprotection in the early post-injury period by reducing lipid peroxidation, owing to three distinctive but chiefly related protective mechanisms (2). These three protective effects of hypothermia have influence on blood supply, cell membrane stabilization, and edema formation (2). Hypothermia reduces the blood supply of traumatized neuronal tissue due to the general vasoconstriction and increased blood viscosity (36). The second beneficial effect of hypothermia is on cell membrane stabilization, which is critical for cell viability after SCI (14,37). Consistent with previous studies about hypothermic protection on lipid peroxidation (6), we measured decreased MDA levels in group D and group F. SCI tended to decrease the tissue levels of GSH-Px, which protects the cell membrane against oxidative damage by regulating the status of proteins in the cell membrane (1). In our study, levels of GSH-Px tended to be increased in groups D and F. In the hypothermia groups (groups D and F), elevated levels of SOD and GSH-Px suggest that the lipid peroxidation process accelerates with increased temperature. The third beneficial effect of hypothermia is on edema formation. Hypothermic implementations have been reported to decrease edema formation by means of reducing the spinal cord metabolism and decreasing protein synthesis (38) when hypothermia was initiated in the earlier post-injury period (39). In the pathological sections of the present study, decreased edema formation was found in hypothermia groups. Results of the neurological examination also supported the efficiency of the hypothermia treatment.

In conclusion, according to our literature search, this is the first study demonstrating the neuroprotective effect of treatment with hypothermia and HBO together after experimental SCI. It seems reasonable to conclude that after acute SCI, combination of hypothermia and HBO protected the spinal cord from the secondary damages through reducing the levels of free radicals by preventing lipid peroxidation. However, molecular mechanisms of HBO and hypothermia treatments are not fully understood. Further studies should be planned, focusing on the ultrastructural pathology and long-term treatment periods. Moreover, additional research is also necessary to further evaluate the potential benefits of these combination therapies in the management of acute SCI injury in humans.

References

1. Serarslan Y, Yönden Z, Ozgiray E, et al. Protective effects of tadalafil on experimental spinal cord injury in rats. J Clin Neurosci 2010;17: 349−352.
2. Duz B, Kaplan M, Bilgic S, et al. Does hypothermic treatment provide an advantage after spinal cord injury until surgery? An experimental study. Neurochem Res 2009;34:407−410.
3. Kurt G, Ergün E, Cemil B, et al. Neuroprotective effects of infliximab in experimental spinal cord injury. Surg Neurol 2009;71:332−336.
4. Görgülü A, Kiriş T, Unal F, et al. Superoxide dismutase activity and the effects of NBQX and CPP on lipid peroxidation in experimental spinal cord injury. Res Exp Med (Berl) 2000;199:285−293.
5. Kalayci M, Coskun O, Cagavi F, et al. Neuroprotective effects of ebselen on experimental spinal cord injury in rats. Neurochem Res 2005; 30:403−410.
6. Tüzgen S, Kaynar MY, Güner A, et al. The effect of epidural cooling on lipid peroxidation after experimental spinal cord injury. Spinal Cord 1998;36:654−657.
7. Braughler JM, Hall ED. Central nervous system trauma and stroke. I. Biochemical considerations for oxygen radical formation and lipid peroxidation. Free Radic Biol Med 1989;6:289−301.
8. Fujimoto T, Nakamura T, Ikeda T, et al. Effects of EPC-K1 on lipid peroxidation in experimental spinal cord injury. Spine 2000;25:24−29.
9. Topsakal C, Erol FS, Ozveren MF, et al. Effects of methylprednisolone and dextromethorphan on lipid peroxidation in an experimental model of spinal cord injury. Neurosurg Rev 2002;25:258−266.
10. Lo TP Jr, Cho KS, Garg MS, et al. Systemic hypothermia improves histological and functional outcome after cervical spinal cord contusion in rats. J Comp Neurol 2009;514:433−448.
11. Gamache FW Jr, Myers RA, Ducker TB, et al. The clinical application of hyperbaric oxygen therapy in spinal cord injury: a preliminary report. Surg Neurol 1981;15:85−87.
12. Nie H, Xiong L, Lao N, et al. Hyperbaric oxygen preconditioning induces tolerance against spinal cord ischemia by upregulation of antioxidant enzymes in rabbits. J Cereb Blood Flow Metab 2006;26: 666−674.

13. Kindwall EP, Gottlieb LJ, Larson DL. Hyperbaric oxygen therapy in plastic surgery: a review article. Plast Reconstr Surg 1991;88:898−908.
14. Tompach PC, Lew D, Stoll JL. Cell response to hyperbaric oxygen treatment. Int J Oral Maxillofac Surg 1997;26:82−86.
15. Kahraman S, Düz B, Kayali H, et al. Effects of methylprednisolone and hyperbaric oxygen on oxidative status after experimental spinal cord injury: a comparative study in rats. Neurochem Res 2007;32: 1547−1551.
16. Rivlin AS, Tator CH. Effect of duration of acute spinal cord compression in a new acute cord injury model in the rat. Surg Neurol 1978;10: 38−43.
17. Westergren H, Farooque M, Olsson Y, et al. Motor function changes in the rat following severe spinal cord injury. Does treatment with moderate systemic hypothermia improve functional outcome? Acta Neurochir (Wien) 2000;142:567−573.
18. Lowry OH, Rosebrough NJ, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265−275.
19. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70:158−169.
20. Carlson SL, Parrish ME, Springer JE, et al. Acute inflammatory response in spinal cord following impact injury. Exp Neurol 1998; 151:77−88.
21. Bulkley GB. The role of oxygen free radicals in human disease processes. Surgery 1983;94:407−411.
22. Demopoulos HB, Flamm ES, Seligman ML, et al. Further studies on free-radical pathology in the major central nervous system disorders: effect of very high doses of methylprednisolone on the functional outcome, morphology, and chemistry of experimental spinal cord impact injury. Can J Physiol Pharmacol 1982;60:1415−1424.
23. Cemil B, Topuz K, Demircan MN, et al. Curcumin improves early functional results after experimental spinal cord injury. Acta Neurochir (Wien) 2010;152:1583−1590. discussion 1590. [Epub ahead of print].
24. Siegal T, Siegal T, Shohami E, et al. Experimental neoplastic spinal cord compression: effect of ketamine and MK-801 on edema and prostaglandins. Neurosurgery 1990;26:963−966.
25. Yashon D, Bingham WG Jr, Faddoul EM, et al. Edema of the spinal cord following experimental impact trauma. J Neurosurg 1973;38: 693−697.
26. Cayli SR, Ates O, Karadag N, et al. Neuroprotective effect of etomidate on functional recovery in experimental spinal cord injury. Int J Dev Neurosci 2006;24:233−239.
27. Ozden TA, Uzun H, Bohloli M, et al. The effects of hyperbaric oxygen treatment on oxidant and antioxidants levels during liver regeneration in rats. Tohoku J Exp Med 2004;203:253−265.
28. Oter S, Edremitlioglu M, Korkmaz A, et al. Effects of hyperbaric oxygen treatment on liver functions, oxidative status and histology in septic rats. Intensive Care Med 2005;31:1262−1268.
29. Ishihara H, Kanamori M, Kawaguchi Y, et al. Prediction of neurologic outcome in patients with spinal cord injury by using hyperbaric oxygen therapy. J Orthop Sci 2001;6:385−389.
30. Yeo JD, Lowry C, McKenzie B. Preliminary report on ten patients with spinal cord injuries treated with hyperbaric oxygen. Med J Aust 1978;2:572−573.
31. Henninger N, Küppers-Tiedt L, Sicard KM, et al. Neuroprotective effect of hyperbaric oxygen therapy monitored by MR-imaging after embolic stroke in rats. Exp Neurol 2006;201:316−323.
32. Rockswold SB, Rockswold GL, Defillo A. Hyperbaric oxygen in traumatic brain injury. Neurol Res 2007;29:162−172.
33. Singhal AB. A review of oxygen therapy in ischemic stroke. Neurol Res 2007;29:173−183.
34. Yin D, Zhang JH. Delayed and multiple hyperbaric oxygen treatments expand therapeutic window in rat focal cerebral ischemic model. Neurocrit Care 2005;2:206−211.
35. LaVan FB, Hunt TK. Oxygen and wound healing. Clin Plast Surg 1990;17:463−472.
36. Knighton DR, Silver IA, Hunt TK. Regulation of wound-healing angiogenesis-effect of oxygen gradients and inspired oxygen concentration. Surgery 1981;90:262−270.
37. Rawe SE, Lee WA, Perot PL. Spinal cord glucose utilization after experimental spinal cord injury. Neurosurgery 1981;9:40−47.
38. Westergren H, Yu WR, Farooque M, et al. Systemic hypothermia following spinal cord compression injury in the rat: axonal changes studied by beta-APP, ubiquitin, and PGP 9.5 immunohistochemistry. Spinal Cord 1999;37:696−704.
39. Dietrich WD. Nonpharmocological strategies—moderate hypothermia. In: Narayan RK, Wilberger JE, Povlishock JT, eds. Neurotrauma. New York: McGraw-Hill; 1996. pp. 1491−1506.