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# Hyperbaric oxygenation as an adjuvant therapy for traumatic brain injury: a review of literature

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## Abstract

*In recent years significant amount of data have been published in the filed of hyperbaric oxygenation (HBO) and traumatic brain injury (TBI). The main rational for the research in this field is that in TBI patients with the existence of dormant neural tissues that maintain cellular homeostasis but are unable to participate in neurotransmission, the addition of HBO provides a favourable environment by which neuronal reactivation can be achieved. As hyperbaric oxygen therapy is not all-or-nothing phenomena and the consequences of TBI can vary from mild to moderate and severe, it is important to evaluate each TBI patient before referring him/her to hyperbaric oxygen therapy (HBOT). Reports from the clinical trial that were investigating the effects of HBO on severe TBI show promising results. For example, significant reduction in mortality rates and improvement in favourable neurological outcomes were reported. However, conflicting results have been reported from trials that investigated the effects of HBO on mild and moderate TBI. The results from the experimental studies indicate that HBO can preserve mitochondrial function, reduce apoptosis and neuroinflammation and promote neuronal plasticity. Therefore, conducting of methodologically-based multicentric clinical trials is necessary to determine proper guidelines for inclusion of TBI patients in HBOT. As many reports*

**List of abbreviations:**

| | |
|---|---|
| ATA | – Atmosphere absolute |
| CT | – Computer tomography |
| CSI | – Cortical stab injury |
| DRS | – Disability Rating Scale |
| DCD | – Dynamic cortical deformation |
| GCS | – Glasgow Coma Scale |
| GOS | – Glasgow Outcome Scale |
| HBO | – Hyperbaric oxygenation |
| HBOT | – Hyperbaric oxygen therapy |
| NBH | – Normobaric hyperoxia |
| PCL-M | – Composite scores on post-traumatic disorder check list-military version |
| PCS | – Post concussion syndrome |
| PCR | – Polymerase chain reaction |
| RLA | – Ranchos Los Amigos Scale |
| SPECT | – Single photon emission computed tomography |
| SCA | – Suction ablation |
| TBI | – Traumatic brain injury |
| TNF-$\alpha$ | – tumor necrosis factor-alpha |
| ImPACT | – Immediate post-concussion assessment and cognitive testing |

*have stated that even a few exposures to HBO can contribute to the recovery process, future research must be aimed at establishing most effective HBO protocol for TBI patients.*

## BACKGROUND

### Traumatic brain injury

Considering the fact that the consequences of traumatic brain injury (TBI) can persist for many years, included for life, TBI can be considered as a chronic disease *(1-4)*. The acute phase is a process that consists of at least two stages. The first is often referred to as a primary injury. It occurs momentarily after the initial mechanical impact and in most of the TBI patients this stage is over before they are admitted to the medical institution. The severity of the primary injury depends on the location of the affected brain region as well as duration and intensity of the inflicted force *(3)*. Biochemical and metabolic processes that follow afterwards in hours, days and sometimes even weeks are referred to as the secondary injury *(4)*. Formation of the glial scar tissue marks the transition of the healing process into the chronic phase *(5)*. Consequences of the TBI can vary from mild, moderate to severe and they can persist for a lifetime. They pose a great personal and a wider socio-economical problem *(6)*.

At site of the primary impact a permanent neuronal loss can be often seen. This region is called the „core". The surrounding regions, consisting of neuronal tissues that have not been directly exposed to trauma, are often addressed to as the „penumbra area". Neurons inside this zone are at risk due to several reasons: impaired blood flow (limited or not at all), inflammation, development of oedema, acidosis, and haemorrhage and they lose most of their connections with other neurons *(7-9)*. Secondary degeneration can also progress into the surrounding intact regions of the brain. Compromised blood flow and insufficient oxygen supply leads to tissue hypoxia and the resulting energy failure initiate a cascade of cellular events that culminates with neuronal cell apoptosis *(10)*.

Most of the neuro-therapeutic strategies are directed towards the containment of the secondary processes and the preservation and reactivation of the penumbra area *(11)*. The activity of glial cells is of tremendous importance in early stages of the healing process, but when the glial scar is formed it represents a barrier for the neuronal sprouting and reduces the ability of brain to re-establish new functional neuronal circuits *(12)*.

Until now universal therapeutic strategy and a single medicament have not been found that are able to affect all the processes that are occurring during TBI and diminish all of its consequences. Considering the fact that energy metabolism in the brain is based upon aerobic processes, it is only logical to assume that in pathological conditions such as TBI, increasing oxygen supply to the traumatized brain tissue should lead to better neurological outcome. The main rational for implementation of hyperbaric oxygen therapy (HBOT) in treatment strategies aimed to improving the consequences of TBI is that in TBI patients addition of oxygen under hyperbaric conditions provides a favourable environment by which neuronal reactivation can be achieved. Unfortunately, even the most sophisticated diagnostic methods are not capable to distinguish with absolute certainty whether the areas that are appear as gliosis may actually be viable nerve tissue that can be reactivated. Single photon emission computed tomography (SPECT) appears to be a most suitable diagnostic method for this task *(13)*.

### Hyperbaric oxygenation mechanism of action

Hyperbaric oxygenation (HBO) or hyperbaric oxygen therapy (HBOT) is the therapeutic modality in which a patient breathes 100% oxygen intermittently, while the pressure of the treatment chamber is increased to more than one atmospheric pressure *(14)*. HBO can be used to obtain 100% saturation of haemoglobin and to significantly elevate the volume of physically dissolved oxygen fraction in blood plasma. HBO also has anti-inflammatory activity and promotes neo-angiogenesis. HBO has become the definitive therapy for patients with decompression illness, air or gas embolism, carbon monoxide poisoning, gas gangrene and compartment syndrome, intracranial abscess, osteomyelitis, delayed radiation injury, skin grafts and flaps, and is a widely accepted treatment for wound healing and burns *(15-16)*.

The exact mechanisms by which HBO exerts its positive effects are still lacking. The rational for application of HBO after TBI is that HBO might produce a marked elevation in arterial blood oxygen partial pressures and content to combat local anoxia and the resulting energy failure, which together initiate a cascade of cellular events that culminate in cell death. In comparison to the normobaric conditions increased oxygen supply under hyperbaric conditions make easier diffusion of oxygen into the damaged tissue and also for longer distance *(17)*. It is assumed that relief of hypoxia and cerebral oedema *(18)*, improvement of microcirculation with decrease of intracranial pressure *(19)*, and improvement of cerebral metabolism *(20)* are also among the positive effects of HBO.

Over the years several systematic reviews have concluded that the evidence for the use of HBO for treatment of TBI is insufficient to prove effectiveness or ineffectiveness, and that more high-quality studies and multicenter prospective randomized clinical trials are needed *(21-24)*. In recent years a substantial amount of new data has been published indicating that HBO can interfere with the processes that follow TBI and moderate its consequences. Accordingly in this review we have focused our attention on finding publications that evaluate this topic in the time period from the year 2008 up to today.

All of the data used in this review were obtained from studies using either human or animal subjects, which could be found in Pub-Med, EMBASE, and the Cochrane Library data base. Combinations of the following keywords were used for the search: traumatic brain injury, hyperbaric oxygen therapy, hyperbaric research, normobaric hyperoxia, adjuvant therapy, cerebral blood flow, cerebral metabolism, intracranial pressure, apoptosis, oxidative stress and oxygen toxicity. All of the studies described here, were reviewed by a local ethics committee and were conducted in accordance to the Declaration of Helsinki (1975) and with recommendations given in NIH Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23).

## Clinical studies

The continuous researches conducted by Sarah Rocksworld and Gaylan Rocksworld alongside with their colleagues will be regarded in the future as milestone studies with respect to implementation of HBO in treatment of severe traumatic brain injury *(25-30)*. In their paper published in *Journal of Neurosurgery* they presented the results of a first prospective, randomized phase II clinical trial aimed at evaluating the effects of combined hyperbaric and normobaric hyperoxia (NBH) in severe TBI *(25)*. Forty-two patients were included in this trial. Research paradigm examined the comparison between combined HBO/NBH treatments with standard care. Twenty patients received the combined HBO/NBH treatment, which consisted of 100% oxygen for 60 minutes at 1.5 atmospheres absolute (ATA), followed by 3 hours at 1.0 ATA. The control treatment for 22 patients was standard care. The first HBO/NBH treatment was administered as soon as the entry criteria were met and the patient's condition was clinically stable. Subsequent treatments were given every 24 hours. Patients received 3 consecutive treatments unless they became brain dead or were consistently able to follow commands. HBO treatment was conducted in multiplace as well in monoplace chamber. There were no statistically significant differences in any of the variables between the patients using the monoplace chamber and those using the multiplace chamber. From this study they concluded that in comparison to standard care combined HBO/NHB treatments significantly improve markers of oxidative metabolism in relatively uninjured brain as well as in pericontusional tissue, reduce intracranial hypertension, and diminish the presence of markers of cerebral toxicity. More importantly they established that mortality rate was statistically significantly lower ($p = 0.0482$, 26% reduction), in the combined HBO/NHB group (16% mortality rate) as compared to the control group (42% mortality rate). Six month after the traumatic incident, favourable outcome as measured by the sliding dichotomized Glasgow Coma Scale (GCS) was observed in 14 out of 19 patients from the HBO/NHB group (74%) and in 8 out of 21 patients from the control group (38%).

The use of combined HBO and NBH therapy was based on the results of their previous study *(26)*. In this study sixty nine TBI patients were within 24 hours of injury, prospectively randomized in to 3 groups: first group received HBO therapy 100% oxygen at 1.5 ATA for 60 minutes, second NBH group received 100% oxygen at 1 ATA for 3 hours, and the control group which have received the standard care. Based on the results, they concluded that HBO has a more robust post treatment effect compared to NHB on oxidative cerebral metabolism. Very important finding was that no signs of pulmonary or cerebral oxygen toxicity were registered. The authors concluded that HBO treatment for severe TBI is not all-or-nothing phenomena and that development of beneficial effects is a gradual process.

For every therapeutic agent adequate time frame for application is of the most importance. Considering the fact that sever TBI represent life threatening conditions and that patients stabilization is a primary goal, one can not expect that such patient will be in a short time period transfer to hyperbaric unite. Therefore it is important to investigate whether delayed application of HBO can exert positive effect on the recovery process.

Lin et al. have reported that sub acute patients with TBI can benefit from HBO with none or minimal side effects *(31)*. In this study they have randomly organized 22 patients who received 100% oxygen at 2 ATA for 2 hours, and the other 22 patients with corresponding conditions into the control group that received standard care. The clinical conditions of the patients were evaluated with the Glasgow Coma Scale and Glasgow Outcome Scale (GOS) before and 3 to 6 months after HBOT. The GCS of the HBO group was improved from 11.1 to 13.5 in average, and from 10.4 to 11.5 ($p < 0.05$) for control group. Among those patients with GOS = 4 before the HBOT, significant GOS improvement was observed in the HBOT group 6 months after the HBOT.

Sahni et al. have conducted a retrospective analysis of TBI patients that were subjected to HBO *(32)*. Although the authors have concluded that the results from their study must be interpreted cautiously, we must address to them as a good starting point for future investigations. The test group consisted of 20 patients that were within the six months after the TBI subjected to HBO. Patients from the test group have all received at least 30 HBO sessions, at 1.5 ATA, for 60 minutes, in multiplace chamber (via oxygen mask, or T-tube), once daily in addition to standard care. Other 20 patients with corresponding clinical characteristics were given only standard treatment. Assessment using the Disability Rating Scale (DRS) showed a significant improvement in the vegetative state among the patients that have received HBO. Thus the patients in the vegetative state (DRS Score 22-24) showed maximum improvement and the percentage dropped from 45% to 5% in test group compared to a reduction from 45% to only 25% in the control group.

The improvement in cognitive functions assessed with Ranchos Los Amigos Scale (RLA) was more prominent in test group, from which 90% patients had a score of ≤ 3 before starting HBO treatment and that was reduced to 35% after treatment. In control group 95% of patients had a similar score before treatment and these was reduced to 60 % after standard treatment.

Hardy et al. have presented a single case study of the therapeutic potential of HBO on chronic brain injury *(33).* A 54-year old man was one year after sustaining severe TBI exposed to 100% oxygen, at 2.0 ATA, for 60 minutes. Initial treatment consisted of 20 exposures, after which improvement in sensory motor functions were observed. Nevertheless, these positive shifts diminished in the following year, but were reinstated with an additional series of 60 HBO exposures. Neuropsychological improvements were also observed after the second treatment series.

Case reports in which positive effects of HBOT on mild TBI are also reported *(34).* However, according to the authors of the first single center, double blind, sham controlled prospective trial in which the effects of HBOT on symptoms after mild TBI were investigated, obtained results were not promising *(35).* At the U.S. Air Forse School of Aerospace Medicine, in 50 military service members, who had sustained at least one combat-related mild TBI, the effects of hyperbaric oxygen on post concussion symptoms were investigated. Participants were organized in two groups. Sham control group was exposed to roam air at 1.3 ATA, and experimental group was exposed to 100% oxygen at 2.4 ATA. Over eight-week period each subject received 30 sessions. Individual and total symptoms scores on Immediate Post-Concussion Assessment and Cognitive Testing (ImPACT) and composite scores on Post-traumatic Disorder Check List-Military Version (PCL-M) were measured just prior to intervention and 6 weeks after completion of intervention. Difference testing of post-intervention means between the sham-control and HBO group revealed no significant differences on the PCL-M composite score ($t = -0.205$, $p = 0.84$) or on the ImPACT total score ($t = -0.943$, $p = 0.35$). PCL-M composite scores and ImPACT total scores for sham control and HBO groups revealed significant improvement over the course of the study for both the sham control group ($t = 3.76$, $p = 0.001$) and the HBO group ($t = 3.90$, $p = 0.001$), demonstrating no significant HBO effect. Paired t-test results revealed 10 ImPACT scale scores in the sham control group improved from pre- to post-testing, whereas two scale scores significantly improved in the HBO group. One PCL-M measure improved from pre- to post-testing in both groups. Based on the results the authors have concluder that that HBO at 2.4 ATA pressure had no effect on post-concussive symptoms after mild TBI.

We must certainly compliment the authors for organizing and completing this complex study. Unfortunately we must also point out serious objections to the study design as other colleagues have already stated *(36, 37).* It has been pointed out that in this study the dose responsive effect of HBO was not taken under the consideration and that sham control group should be considered to be also a treatment group and that the conclusion of the authors that HBO does not exerts any positive effective on mild brain injury is at this point an overstatement.

Researches conducted by Harch and his colleagues have also focused the attention on this topic *(38).* The first phase study of low pressure HBOT for blast induced post concussion syndrome (PCS) and post traumatic stress disorder (PTSD) was conducted on sixteen military subjects with chronic mild to moderate traumatic brain injury. The HBO protocol consisted of 100% oxygen at 1.5 ATA for 60 min, 40 sessions in 30 days. According to the obtained data they have concluded that significant improvements occurred in symptoms, abnormal physical exam findings, cognitive testing, and quality-of-life measurements, with concomitant significant improvements in results from the SPECT imaging.

Recently published results of Boussi-Gross et al. have demonstrated the effectiveness of HBOT in improving brain function and quality of life in mild TBI patients suffering from chronic neurocognitive impairments *(39).* The trial population included 56 mild TBI patients that within one to five years after sustaining brain injury still hade prolonged post concussion syndrome. The HBO effects were evaluated by means of prospective, randomized, crossover controlled trial. The patients were randomly assigned to treated or crossover groups. Patients in the treated group were evaluated at baseline and following 40 HBO sessions. Patients in the crossover group were evaluated three times: at baseline, following a two-month control period of no treatment, and following subsequent two months of 40 HBO sessions. The HBO protocol included 40 treatment sessions, five sessions per week, 60 minutes each, with 100% oxygen at 1.5 ATA. "Mindstreams" was used for cognitive evaluations, quality of life was evaluated by the EQ-5D questionnaire, and changes in brain activity were assessed by SPECT imaging. Significant improvements were demonstrated in cognitive function and quality of life in both groups following HBOT but no significant improvement was observed following the control period. SPECT imaging revealed elevated brain activity which was in good agreement with the cognitive improvements. Their conclusions were that HBOT can induce neuroplasticity leading to repair of chronically impaired brain functions and improved quality of life in mild TBI patients with prolonged PCS at late chronic stage.

These results are raising the most significant question and that is how to properly select TBI patients for enrolment in HBO treatment. Patient whit the existence of „idle" neurons in other words „dormant" neural tissue that maintains cellular homeostasis but is unable to participate in neurotransmission is, as it was correctly stated in these studies, the one that can expect the most benefit from

HBOT. The main pathology in these cases involves diffuse brain injuries, which are hard to detect by anatomical imaging yet noticeable in metabolic imaging. Accordingly, the existence of the discrepancy between the results from the „metabolical" (such as SPECT scan) and „anatomical" (such as CT) imaging techniques should be focused upon, in an effort to properly determine the possibility for achieving the most beneficial effects of HBOT in TBI patients.

At this point it must be stated that in conducted studies the development of significant adverse effects, especial in terms of oxidative damage in TBI patients who were exposed to hyperbaric oxygen were not registered. Cases of sporadic middle ear barotraumas and reversible bronchospasm were reported.

Undergoing research regarding the effects of hyperbaric oxygen on post-concussion syndrome organized by The Department of Defence of United States of America, in collaboration with the Department of Veterans Affairs will, as they have stated comprehensively examine the potential for the use of HBO in treatment of mild TBI *(40).* They are planning four randomized controlled trials which will enrol a total of 242 service members with post-concussion syndrome. Participants will be exposed to a range of control, sham and HBO conditions for 40 sessions over a period of eight to eleven weeks. Compression pressures will range from 1.2 ATA (sham) to 2.4 ATA, and oxygen concentration will range from room air (sham and control) to 100%. Outcomes measures will include both subjective and objective measures performed at baseline, at exposure completion, and at three to twelve months' follow-up. As they have stated, this integrated program of clinical trials investigating the efficacy of HBOT in service members with persistent symptoms following mild TBI exposure will be important to define practice guidelines and, if needed, for the development of definitive clinical trials in this population.

## EXPERIMENTAL STUDIES

One can only speculate whether the same results as the ones obtained from the studies conducted on animal models, can be expected in studies conducted on human population. But still animal models of TBI can give us very useful information and present guidelines for future research design in humans. Since impaired control of locomotor functions is one of the most severe consequences of TBI, many animal studies are directed to test whether HBOT can contribute to this vitally important aspect of the recovery process.

Lim et al. have reported that implementation of HBO can contribute to the recovery of motor skills in rats after TBI, and that potential underlining mechanism is attenuation of neuroinflammation response *(41).* Fluid percussion technique was used to inflict TBI in rats. Hyperbaric protocol consisted of a single exposure to 100% oxygen, at 2.0 ATA, one hour or eight hours subsequent to TBI. Neurobehavioral rehabilitation was evaluated by the inclined plane test on the 72 hour after TBI. The following parameters were also evaluated: infarction area, neuronal apoptosis, microglial cell aggregation count, and tumor necrosis factor-alpha (TNF-α) expression in microglia cell. They have registered that regardless of whether the animals were treated with HBO one or eight hours after TBI they have exhibited significantly better scores in behavioural tests in comparison to the control animals. Cerebral infarction areas of the rats after TBI were significantly attenuated by HBO therapy compared with the controls. TBI induced microglial activation, TNF-α expression, and neuronal apoptosis were also significantly reduced by HBO therapy. Their results demonstrate that HBO treatment during the acute phase of TBI can posses a neuroprotective effect and that attenuation of microgliosis and reduction of proinflammatory cytokine TNF-α expression are among the underlining mechanisms.

In our studies we have used two different models of experimental TBI. In order to examine the effects of HBO upon recovery of motor function we have used suction ablation model (SCA), and in an effort to comprehend underlining mechanisms we have used cortical stab injury (CSI) model of TBI (42-44). Suction ablation model of TBI inflicts unilateral highly reproducible lesions of hindlimb sensorimotor cortex, of uniform size and depth (45). The first exposure to HBO occurred when all the animals have regained consciousness five hours after the operation. HBO protocol consisted of 60 minute exposure to 100% oxygen at 2.5 ATA, which was continued once a day for 10 consecutive days. For evaluation of the recovery process beam walking and grip strength behavioural testing were conducted. The effects of SCA and HBOT on expression profiles of markers of synaptic plasticity, growth-associated protein 43 and protein involved in synapse formation - synaptophysin, were detected using immunohistochemitry. We have registered that HBOT can improve locomotor ability in rats after TBI and that intensified neuronal plasticity in terms of enhanced axonal sprouting and synapse remodelling are among the underlining mechanisms.

As the role of reactive oxygen species (ROS) in induction of neuronal damage and prevention of neuronal plasticity is becoming more apparent, a great interest was put on investigation of the effect of HBOT on parameters of antioxidant defence mechanisms after TBI *(46, 47).*

Studies conducted by Palzur, Voldavsky and their colleagues have clarify many underlining mechanisms in these field *(48-52).* Most of their research was conducted on dynamic cortical deformation model (DCD) of TBI. HBO protocol that they have used consisted of two consecutive 45 minute sessions, during which 100% oxygen was administered at 2.8 ATA. First HBO exposure started 3 hours after the injury and second one 24 hours later. Theirs earlier research have shown that HBO can reduce secondary neuronal degeneration and can attenuate apop-

tosis after experimental TBI *(48)*. They have also reported that HBO can reduce neuroinflammation after TBI *(49)*. The preservation of mitochondrial function and integrity, as well as the reduction of oxidative damage, are according to their results among the most important effect of HBO in the recovery process after TBI *(50)*. Therefore, they have proven the most commonly used rational for the research in the field of implementation of HBO in treatment strategies intentional to improve the consequences of TBI, and that is that increased intracellular oxygen bio-availability can both contribute to preserve mitochondrial integrity and reduce the activation of the mitochondrial pathway of apoptosis. Among the animals that underwent HBO protocol there was a significant attenuation of the impact of DCD over perilesional neurons, characterized by significantly higher cell counts and denser axonal network. In mitochondria isolated from injured brain tissue, HBO substantially reversed the loss of mitochondrial transmembrane potential. Their reports are stating that HBO can selectively affect the intrinsic pathway of apoptosis *(51, 52)*.

Zhou et al. have also showed that protection of mitochondrial function after TBI is one of the most important effects of HBOT *(53)*. They have reported that the improvement of cognitive functions was registered in rats that were exposed to HBO. They have used lateral fluid percussion model of TBI and a variety of both HBO and NBO protocols in their research.

Our group has also observed that reduction of oxidative stress can be achieved by application of HBOT after TBI *(43)*. Cortical stab injury model was used in this research. HBO protocol consisted of 60 minute exposure to 100% oxygen at 2.5 ATA, once a day for 3 or 10 consecutive days. Pattern of superoxide dismutase 2 (SOD2) expression and cellular localization were analysed using Real-time PCR, Western Blot and double-label fluorescence immunohistochemistry. Neurons undergoing degeneration were visualized with Fluoro-Jade. Our results have indicated that injury induced significant transient increase in SOD2 protein levels at day 3 post injury, which was followed by reduction towards control levels at post-injury day 10. At the same time points, mRNA levels for SOD2 in injured cortex were down-regulated. Exposure to HBO for 3 days considerably down-regulated SOD2 protein levels in the injured cortex, while after 10 days of HBOT an up-regulation of SOD2 was observed. HBOT significantly increased mRNA levels for SOD2 at both time points compared to corresponding group. Double immunofluorescence staining revealed that 3 days after the TBI up-regulation of SOD2 was mostly due to increased expression in reactive astrocytes surrounding the lesion site. HBOT attenuated SOD2 expression both in neuronal and astroglial cells. According to Fluoro-Jade B labelling, HBOT decreased the number of degenerating neurons in injured cortex. So, we have concluded that HBOT can moderate the effect of oxidative stress after TBI by influencing expression pattern of SOD2 and thereby reduces neuronal degeneration. Our preliminary data are showing that HBO can also interfere with the activity of glial cells after TBI, especially astorcytes and microglia, so in the future we will focus to investigate that in more details *(44)*.

In their complex study Wang *et al. (54)* have used Feeney model of TBI *(55)*. The right cerebral cortex of rats was injured by the impact of a 20-g object dropped from a predetermined height. Neurological behaviour score, brain water content, neuronal loss in the hippocampus, and cell apoptosis in the brain tissue surrounding the primary injury site were examined in order to determine brain damage severity. In an effort to determine most effective time frame for the application of HBO, animals were exposed to a single treatment with 100% oxygen, at 3 ATA, for 60 minutes, at the following time points: 3, 6, 12, 24, 48 and 72 hours after TBI. Three and six hours after TBI, HBO-treated rats displayed a significant reduction in brain damage. However, by 12 hours after TBI, the efficacy of HBO treatment was considerably attenuated. Furthermore, at 24, 48, and 72 hours after TBI, a singl HBO treatment did not show any notable effects. The following experiments evaluated the effects of multiple exposures to HBO (three or five times in all) that started at the same time points. The results have shown that even when implementation of HBO started 48 hours after TBI it can remarkably reduced neurological deficit scores and the loss of neurons in the hippocampus. The overall most prominent beneficial effects were registered after a single treatment at 6 hours after TBI. Based upon these results authors have concluded that within 12 hours after TBI even a single HBO treatment could alleviate brain damage and that multiple HBO treatments have the possibility to extend the post-TBI delivery time window. This research group has also reported that HBO can influence the activity of glial cells, oxidative status and inflammatory processes after TBI *(56, 57)*. Unfortunately for us these papers were *in extenso* published in Chinese language.

Several studies have used the magnetic resonance imaging (MRI) to monitor the effects of HBOT on experimental TBI. Based on the analysis of their results Wei et al. have concluded that 10 HBO exposures could improve the impaired blood brain barrier and cytotoxic oedema in rabbits after TBI *(58)*. On the other hand, Voigt et al. have shown that even a single exposure to hyperbaric oxygen starting 1 h after impact injury significantly attenuated lesion growth and had long-lasting neuroprotective effects on the contused brain and its penumbra *(59)*. Harcha et al. have demonstrated that the 40-day series of 80 low pressure HBOT can improve behavioural and neurobiological outcome in rats after unilateral focal cortical contusion *(60)*. They have registered an increase in contused vascular density which was associated with improvement in cognitive functions.

Recent publication by Chor et al. has indicated that the addition of inert gases to HBO sessions, especially argon or xenon which show neuroprotective experimental effects, may provide an additional improvement of cerebral lesions *(61).*

## CONCLUSION

Our opinion is that the critical amount of data have been published during recent years that are justifying future clinical trials in the filed of hyperbaric oxygenation and traumatic brain injury. Further multicentric studies must be strictly methodologically organized in order to come up with adequate answers to the following questions: how to make a proper selection among TBI patients for inclusion in hyperbaric oxygen therapy, when to start with HBO exposures after TBI, which pressure to apply, duration and the adequate number of sessions, and can the application of normobaric hyperoxia be sufficient enough to achieve positive neurological outcome after traumatic brain injury.

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