Journal of Animal and Veterinary Advances 13 (17): 1045-1051, 2014
ISSN: 1680-5593

Effect of Electroacupuncture on Transcutaneous Oxygen Pressure During Hyperbaric Oxygen Treatment in Traumatic Brain Injury Patients

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Abstract: The present study investigates if Electroacupuncture (EA) during Hyperbaric Oxygen Treatment (HBOT) has greater effect in Traumatic Brain Injury (TBI) patients than HBO or EA alone. Thirty patients were randomized into three groups: a Hyperbaric Oxygen (HBO) group (n = 10) an EA group (n = 10) and an EA during HBO group (n = 10). Before, during and after EA treatment, variations in Transcutaneous oxygen Pressure ($TcPO_2$) were monitored in each group. Before and after treatment, variations in Glasgow Coma Scale score (GCS) were monitored in each group. During EA treatment, the increase in $TcPO_2$ among subjects in the EA during HBO group was significantly greater than that observed among subjects in the HBO and EA groups ($p<0.01$). After EA treatment, the average $TcPO_2$ level in the EA during HBO group remained significantly higher than that observed in the other two groups ($p<0.01$). Moreover, the total $TcPO_2$ level measured in the EA during HBO group was significantly higher than that of the other two groups ($p<0.01$). After treatment, the GCS level measured in the EA during HBO groups was significantly higher than that of the other two groups ($p<0.01$) and the total GCS level measured in the EA during HBO group was significantly higher than that of the other two groups ($p<0.01$). The therapeutic effect of EA during HBO on TBI was greater than that of HBO or EA alone.

Key words: Electroacupuncture, hyperbaric oxygen treatment, traumatic brain injury, hyperbaric oxygen, transcutaneous oxygen pressure, glasgow coma scale score

INTRODUCTION

Thegoal of Hyperbaric Oxygen Treatment (HBOT) is to significantly increase the oxygen (O_2) supply to the body. During HBOT, the O_2 levels are increased to a greater extent than the normal pressure achieved and this increase in O_2 is then delivered throughout the body to improve the anoxic state.

Traumatic Brain Injury (TBI) is caused by traffic accident, high altitude dropping, falling down and war. It is one of familiar wound which threaten human life and affect quality of life. HBO therapy can significantly decrease disability and fatality rate. Early application of HBO therapy for TBI can prevent disease progression and obtain good long-term effect (Tao *et al*., 2011).

HBO therapy aims to increase the levels of physically dissolved oxygen and tissue oxygen pressure to remedy hypoxia and promote aerobic metabolism. Arterial oxygen Pressure (PaO_2) is proportional to the environmental pressure and each environmental pressure corresponds to a fixed maximum value of PaO_2 because of the toxic side effects and complications of HBOT, the environmental pressure and treatment time must be restricted. This value of the most common treatment pressure for Hyperbaric Oxygen (HBO) in adults can range from 2.0-2.5 Atmospheres Absolute (ATA). So, we hypothesized that other therapy applied during HBOT may safely improve the value of PaO_2 during HBOT in TBI patients and produce a greater the rapeutic effect.

Acupuncture is a type of traditional Chinese medicine therapy and has been used as a clinical treatment for 2000 years in China. According to the meridian acupoint, acupuncturists utilize different acupuncture needles and acupuncture manipulations to regulate body function and prevent and treat disease.

Electroacupuncture (EA) is a new method of therapy that combines electricity with acupuncture. After acupuncture of the acupoint, the acupuncture needle body is electrified via the specific electric current in the retaining needle. Stimulation of the retaining needle is combined with the electric current to strengthen the stimulation of the needle. EA demonstrates a stimulatory effect similar to persistent needle manipulation, although, the stimulus parameters can be objective. The EA apparatus is the primary medication tool of EA therapy and the variations in waveform, frequency and

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intensity of the electric current in the EA apparatus deliver different therapeutic effects. Acupuncture therapy has many advantages such as its use for a wide variety of indications; improved therapeutic results that are delivered rapidly; lower treatment cost; fewer side effects and increased effectiveness compared with other therapies. The selected acupoints are commonly used and they demonstrate good efficacy in the treatment of cardiovascular and cerebrovascular diseases can dredge the meridian and can promote blood circulation.

Literature reviews suggest the effect of the acupuncture on TBI is good (Zhou *et al*., 2011; Wang *et al*., 2012; Zollman *et al*., 2012; Kim *et al*., 2013; Shih *et al*., 2013; Tseng *et al*., 2013; Zhang *et al*., 2013a, b). EA intervention at early stage can promote the recovery of neurological function, accelerate the consciousness from coma and improve the outcomes of patients with severe traumatic brain injury (Zhang *et al*., 2013a, b). Patients with TBI who receive acupuncture treatment have reduced the use of emergency care and hospitalization in the 1st year after injury (Shih *et al*., 2013). Acupuncture can improve neuranagenesis by promoting the proliferation and differentiation of neural stem cells in brain tissues (Zhang *et al*., 2013a, b). EA preconditioning reduces cerebral ischemic injury via BDNF and SDF-1α in mice (Kim *et al*., 2013). Acupuncture helps regain post operative consciousness in patients with traumatic brain injury (Tseng *et al*., 2013). EA pretreatment protects the brain against cerebral ischemic injury (Wang *et al*., 2012). EA increased cerebral blood flow and reduced ischemic brain injury (Zhou *et al*., 2011). Acupuncture has a beneficial effect on perception of sleep or sleep quality and on cognition in the small sample of patients with TBI (Zollman *et al*., 2012).

Transcutaneous Oxygen Pressure ($TcPO_2$) is measured using a noninvasive diagnostic technique in which the partial pressure of oxygen is recorded at the skin surface. When special electrodes (CLARK electrodes) are placed on heated skin, O_2 diffuses from the capillaries into the subcutaneous tissue and skin and electrodes monitor this value as the $TcPO_2$. This measurement provides information regarding the functional status of capillaries and the oxygen supply to the major circulation. Furthermore, in adults, this measurement can be applied in wound evaluation, hyperbaric therapy, plastic surgery, amputation level determination and peripheral vascular disease assessment (such as for limb revascularization procedures). The values of $TcPO_2$ are ~10% less than those of PaO_2, although, the coefficient correlation is 0.99 (Weaver, 2007).

Significantly increased $TcPO_2$ values have been observed in regions surrounding acupoints (Hong *et al*., 2012). Furthermore, powerful stimulation acupuncture (EA reinforcement manipulation) or an increased needle retention time (≥20 min) could significantly increase the $TcPO_2$ of acupoints along the same meridian (Yu *et al*., 1996; Wang *et al*., 1997; Gong *et al*., 2011).

Glasgow Coma Scale (GCS) is the most widely used and validated tool to evaluate the state of consciousness after brain injury. It is devised as a formal scheme to overcome the ambiguities that arose when information about comatose patients was presented and groups of patients compared. The GCS provides a score in the range 3-15; patients with scores of 3-8 are usually said to be in a coma.

Therefore, we hypothesized that EA therapy applied during HBOT may further safely improve the value of $TcPO_2$ (PaO_2) and improve GCS score during HBOT and produce a greater therapeutic effect. No similar studies regarding EA during HBOT have been published to date.

MATERIALS AND METHODS

This prospective, randomized controlled study was conducted after receiving approval from the institutional research and ethical committee of the 401st Hospital of the Chinese People's Liberation Army (PLA). The study protocol was applied to subjects between the ages of 18 and 75 years (average age, 41.8±5.6 years). The study included 14 males and 16 females for a total of 30 TBI patients.

Inclusion criteria:

- Within three days after injury
- Subject was a male or female between the age of 18 and 75
- The definitive diagnosis of subject was made by CT or MRI test
- Subject had stable vital signs
- Subject had no other serious complications except brain injury
- Subject' GCS was 9-12
- Subject had an adequate nutritional status

Exclusion criteria:

- Subject had underwent multiple injuries
- Subject had suffered serious cardiopulmonary disease (e.g., bulla, untreated pneumothorax and mediast inale mphysema, tuberculosis cavities and hemoptysis, severe emphysema, severe upper respiratory infection, bronchiectasis, above second degree atrioventricular block)
- Subject had suffered from hematological diseases
- Subject had suffered from amotio retinae
- Subject was in early pregnancy (within 3 months), post-partum and period

There were no significant differences in gender, age and GCS pre-treatment among the three groups according to the homogeneity test of variance and χ^2-test. Written informed consent was obtained from all subjects. The subjects were randomized using computer-generated random numbers into three groups: HBO group (n = 10), EA group (n = 10) and EA during HBO group (n = 10).

According to the M. Kendall principle, the sample size estimations for multi-factor analysis indicated that the number of each sample should be at least 5-10 times greater than the number of variables. The study included only two variables and 30 subjects.

The stimulated acupoints in the upper extremities of the subjects included PC6 (neiguan) and PC4 (ximen) and the measured acupoint was PC3 (quze). The stimulated acupoints in the lower extremities of the subjects were SP6 (sanyinjiao) and SP8 (diji) and the measured acupoint was SP9 (Yinlingquan). First, the subjects were placed in the supine position and the homolateral PC6 and PC4 acupoints of the subject were perpendicularly pierced to a skin depth of 1 cm (0.25×25 mm; HWATO, Suzhou, Medicine Instrumentation Inc., Suzhou, China). Second, in the EA group, the wire clips of the EA Instrument (SDZ-II, Schow Medicine Instrumentation Inc., Suzhou, China) were linked to the needles of the PC6 and PC4 acupoints. Third, using a selected continuous wave of 3 Hz and pulse amplitude of 3 mA, the needles were retained in the skin for 15 min. For acupuncture of the PC6 and PC4 acupoints of the subjects, the $TcPO_2$ microsensor (QSG-1000B Monitor; Beijing Qiumanshi Technology Inc., Beijing, China) was positioned above the skin of the PC3 acupoint. For acupuncture of the SP6 and SP8 acupoints of the subjects, the $TcPO_2$ microsensor was positioned above the skin at the SP9 acupoint. $TcPO_2$ was recorded each minute using a matched electrochemical analyzer. Because there was only one $TcPO_2$ sensor, each experiment only tested the $TcPO_2$ on one side of the body. Testing was conducted from the left side to the right side and from the upper limbs to the lower limbs.

In the EA during HBO group, HBO was administered at 2.2ATA in a hyperbaric chamber (Yantai Moon Co., Ltd. Gas Compression Equipment Branch Company, Yan tai, China). The total duration was 95 min and the compression time was 15 min. When the pressure reached 2.2ATA, the stabilizing pressure was maintained for 60 min with the subjects breathing pure O_2. The decompression time was 20 min. During the pressure stabilization period, the subjects breathed pure O_2 for 15 min. When the measured $TcPO_2$ reached a steady state, researchers continuously recorded the $TcPO_2$ value for the measured acupoint for 15 min (pre-EA treatment data). Then, we initiated EA with stimulated acupoints; the needles were retained for 15 min during which time we continuously recorded the $TcPO_2$ of the measured acupoint (on-going EA data). When EA was completed, researchers continuously recorded the $TcPO_2$ of the measured acupoint for 15 min (post-EA treatment data) (Fig. 1).

During the pressure stabilization period, subjects in the HBO group breathed pure O_2 for 15 min. When the measured $TcPO_2$ reached a steady state, we continuously recorded the $TcPO_2$ of the measured acupoint for 45 min. The $TcPO_2$ data of the measured acupoint during the second 15 min period corresponded to the pre-EA treatment control group; the $TcPO_2$ data of the measured acupoint during the third 15 min period corresponded to the on-going EA control group and the $TcPO_2$ data of the measured acupoint during the fourth 15 min period corresponded to the post-EA treatment control group (Fig. 2).

For the EA group, except for the high level of atmospheric pressure, the environmental conditions of the laboratory were the same as those in the experimental hyperbaric chamber. When the measured $TcPO_2$ reached a steady state, we continuously recorded the $TcPO_2$ of the measured acupoint for 15 min (pre-EA treatment data). Then, EA was performed at stimulated acupoints with the needles retained for 15 min. At the same time, we continuously recorded the $TcPO_2$ of the measured acupoint (on-going EA treatment data). When EA was completed, we continuously recorded the $TcPO_2$ of the measured acupoint for 15 min (post-EA treatment data) (Fig. 3).

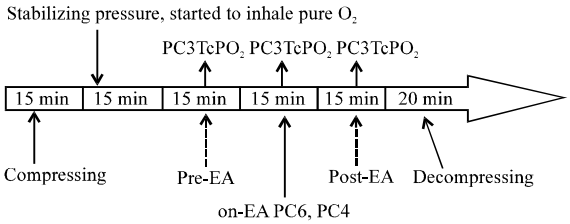

Fig. 1: The EA during HBO group

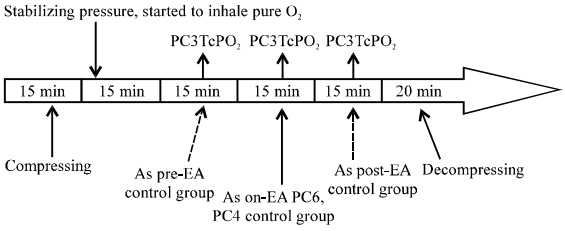

Fig. 2: The HBO_2 group

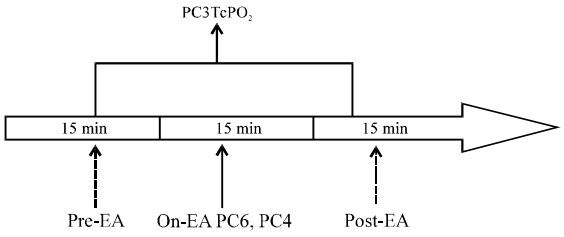

Fig. 3: The EA group

Evaluation of side effects: The side effects of HBOT primarily included barotrauma to the otic, sinus and pulmonary systems. The side effects of EA included bent needles, stuck needles, broken needles, fainting and subcutaneous hemorrhage. During the study, the researchers closely monitored the treatment response of the subjects. If any subject felt unwell, we immediately stopped the study and took appropriate measures to ensure subject safety.

Date processing and filtering: During this study, abnormal data resulting from experimental instrument malfunction, sudden changes in environmental conditions in the laboratory, oxygen inhalation interruption, EA or HBO intolerance of the subjects and side effects were rejected.

Data and statistical analysis: Two researchers inputted the experimental data twice and checked the entries twice. Because acupuncturists had to perform the needling, we performed a double-blind analysis of the subjects and data. The data of each acupoint that targeted five times were averaged and the data of acupoints in the upper and lower extremities of each subject were averaged. Descriptive data are presented as the means±Standard Deviation (SD). The control study for the pre-EA treatment on-going EA and post-EA treatment in each group and pre and post-treatment in each group were performed. The repeated measures, multivariate analysis of variance, paired t-test and one-way analysis of variance were performed using the SPSS17.0 Statistical Software Package. The $p<0.05$ were considered statistically significant.

RESULTS

Table 1 and Fig. 4 showed during EA showed during EA, the increase in $TcPO_2$ among subjects in the EA during HBO group was significantly greater than that observed among subjects in the other two groups

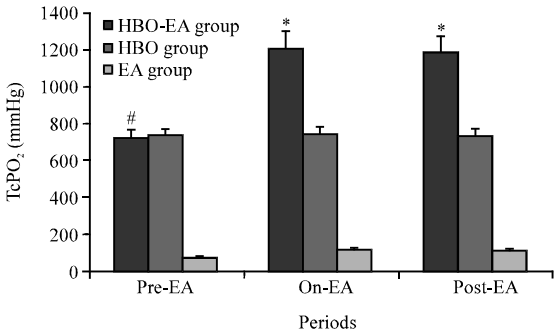

Fig. 4: Comparison of $TcPO_2$ at different periods among the three groups; #: compared with HBO group $p>0.05$; *: compared with HBO group $p<0.01$

Table 1: Comparison of $TcPO_2$ at different periods among the three groups (mmHg, mean±SD)

Groups	Case	Pre-EA	On-EA	Post-EA
HBO+EA	10	726.5±16.3[a]	1208.3±17.2[be]	1191.8±19.9[be]
HBO	10	738.8±21.3	751.4±18.9	742.7±23.2
EA	10	5.2±7.90	117.6±8.50[c]	111.5±9.60[c]

[a]Compared with HBO group $p>0.05$; [b]compared with HBO group $p<0.01$; [e]compared with pre-EA treatment $p<0.01$ and [c]compared with pre-EA treatment $p<0.05$

Table 2: Comparison of GCS at different periods among the three groups (mean±SD)

Groups	Case	Pre-treatment	Post-treatment
HBO+EA	10	9.8±1.1[a]	14.2±0.8[be]
HBO	10	9.5±0.9	12.6±0.7[e]
EA	10	9.6±1.2	10.9±1.4[c]

[a]Compared with HBO group $p>0.05$; [b]compared with HBO group $p<0.01$; [e]compared with pre-treatment $p<0.01$ and [c]compared with pre-treatment $p<0.05$

($p<0.01$). After EA, the average $TcPO_2$ level in the EA during HBO group remained significantly higher than that observed in the other two groups ($p<0.01$). In the EA during HBO group during and after EA, the average $TcPO_2$ level significantly higher than that observed at pre-EA period ($p<0.01$). In the EA group, during EA, the average $TcPO_2$ level significantly higher than that observed at pre-EA period ($p<0.01$). At three periods, the average $TcPO_2$ level in the HBO group remained small fluctuation ($p>0.05$). Before, during and after EA, the average $TcPO_2$ level in the HBO group was significantly greater than that measured in the EA group ($p<0.01$).

Table 2 and Fig. 5 showed after treatment, the GCS level measured in the three groups were significantly higher than before treatment ($p<0.01$). And the GCS level measured in the EA during HBO group was significantly higher than that of the other two groups ($p<0.01$). Moreover, the total GCS level measured in the EA during HBO group was significantly higher than that of the other two groups ($p<0.01$).

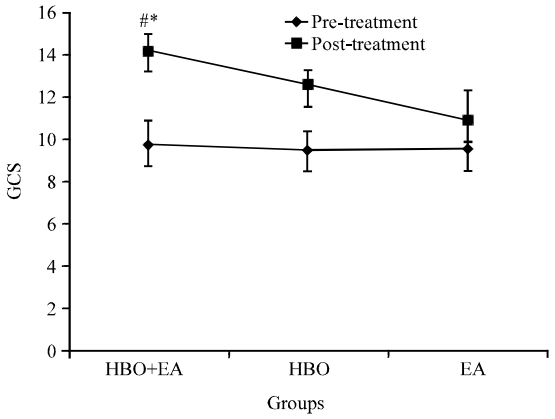

Fig. 5: Comparison of GCS at different periods among the three groups; #: compared with HBO group $p<0.01$; *: compared with pre-treatment $p<0.01$

DISCUSSION

The study shows that although HBOT or EA alone could increase the $TcPO_2$ level in treated subjects, the average $TcPO_2$ level in the EA during HBO group was significantly higher than that in the other two groups. The drop in $TcPO_2$ observed in the EA during HBO group was reduced after EA treatment. And the total $TcPO_2$ level of the EA during HBO group were significantly higher than that of the other two groups. After treatment, the GCS of the EA during HBO group were significantly higher than that of the other two groups. These results show that the therapeutic effect of EA during HBO on TBI was greater than that observed HBO or EA alone.

The higher PaO_2 was more tissue blood oxygen content was. Then, it is the stronger to repair brain tissue and peripheral nerve injured, increase diffusion distance of blood oxygen, develop collateral circulation and ensure neurotrophin supply. So, the therapeutic effect of TBI was better.

An increase in therapy pressure increases the risk of organism injury caused by high pressure. However, the study showed that a significantly increased PaO_2, without an increase in therapy pressure not only further increased the therapeutic effect of HBO but also reduced the incidence of pressure injury caused by HBO. According to Henry's law when the temperature is constant, the quantity of gas dissolved in a solution is proportionate to the partial pressure of the gas. Within a certain pressure range of HBO, higher PaO_2 levels are associated with greater quantities of O_2 dissolved in the plasma which results in a superior therapeutic effect of HBO. Accordingly, the study indicated that EA during HBO while ensuring an effective therapy level of PaO_2 can reduce the therapy pressure of HBO as well as pressure injury caused by HBO.

Although, the increase in PaO_2 increased the risk of oxygen toxicity, this type of toxicity can also result from breathing O_2 too long. During the study, the duration of O_2 breathing was maintained at 60 min. When a healthy individual with a PaO_2 of 250 kPa (1875 mmHg) breathes pure O_2 for ~4 h their vital capacity will decrease by ~50% when they breathe pure O_2 for >5 h, 10% of subjects will demonstrate symptoms of central nervous system oxygen toxicity and when they breathe pure O_2 for 60 min, the Unit Pulmonary Toxic Dose (UPTD) of the subjects is 190.49. When conventional HBO treatment is applied, the UPTD of subjects should not exceed 615. The UPTD of the subjects was significantly below 615 which demonstrates that the protocol was safe.

For higher PaO_2 values there will be greater quantities of O_2 dissolved in the plasma and when there is increased O_2 dissolved in the plasma, the PaO_2 level will be greater. Therefore, researchers assumed that the cause of the PaO_2 increase was the increased quantity of O_2 dissolved in the plasma. When the temperature and pressure are kept constant, this quantity of O_2 dissolved in the plasma will increase due to vasodilatation and increased blood flow. Accordingto previous reports, acupuncture can dilate the vascular system and increase blood flow (Yu *et al*., 1996; Wang *et al*., 1997; Gong *et al*., 2011) and it may partially neutralize the vasoconstriction induced by HBO, thereby dilating the vascular system and increasing the levels of blood flow and PaO_2. Acupuncture can increase the NO level within the treatment region and increase local blood circulation. Moreover, the NO synthesized by endothelial Nitric Oxide Synthase (eNOS) leads to secondary vasodilatation. In particular, EA can prevent the decrease in NO synthesis by eNOS and even stimulate the high expression of eNOS (Tsuchiya *et al*., 2007; Kimura *et al*., 2013). Furthermore, acupuncture of the ST36 (zusanli) acupoint can inhibit the increased expression of neuronal Nitric Oxide Synthase (nNOS) and inducible Nitric Oxide Synthase (iNOS) (Kim *et al*., 2006). EA was also shown to decrease the expression of nNOS and iNOS (Yang *et al*., 2000; Jang *et al*., 2003) and another study reported that EA increased expression of eNOS during hypoxia (Pan *et al*., 2010). Thus, these results may support the use of EA during HBO.

Many experiments showed acupunctcure can remit cerebral vasospasm, improve blood flow around the site of the injury and induce effective collateral circulation. Thus, it can alleviate brain edema and intracranial hypertension and promote brain cell metabolism. acupunctcurecan stimulate the recovery of cerebrovascular autoregulatory mechanism injuried and improve neurological function recovery.

HBOT and EA were combined in the study to get a certain synergistic effect. It was not the simple sum of the two therapeutic effects. On the one hand, HBOT made the body's tissues get enough oxygen and the stimulation of EA can dredge the channel, dilate blood vessels and increase blood flow. On the other hand, the mechanical compression of HBO increase stimulating intensity of EA and reinforce therapeutic effects of EA. The therapeutic effect of EA during HBO was significantly greater than that observed HBO or EA alone.

The major obstacles to EA in a hyperbaric oxygen chamber include percutaneous puncture and the power of EA. However, for current HBO therapies, the technologies related to percutaneous puncture and the power and pressure-retaining medical instruments in the hyperbaric oxygen chamber have improved. As a result, the treatment technology for EA during HBO is practical and safe.

One of the limitations of traditional Chinese acupuncture treatment is that it is human-operated which can lead to artificial influences. However, the EA treatment in the study largely avoided this influence of manual operation because the waveform, magnitude and frequency of the current used for EA treatment were controlled with the EA instrument. Nevertheless, although, we used a fixed needle depth, the insertion of the acupuncture needle was performed by an acupuncturist which artificially influenced the results. However, the methods were designed to mitigate this influence as much as possible in particular, only one acupuncturist executed the entire treatment course and each acupoint was targeted 5 times.

The previous clinical trials showed that when subjects breathed pure O_2 during the pressure stabilization period of HBO their $TcPO_2$ levels gradually increased. After 10-15 min, the $TcPO_2$ reached a steady state and $TcPO_2$ fluctuated within a small range without interference. The application of EA during HBO was not initiated until the $TcPO_2$ reached a steady state and the pressure stabilization period during which pure O_2 was breathed was 60 min. Moreover, the application of EA during HBO began after the subjects had breathed pure O_2 for 15 min at the stabilizing pressure for standardization purposes. Before each experiment began, the $TcPO_2$ test instrument was adjusted according to the regulations of the manufacturer to guarantee that the $TcPO_2$ test instrument was working properly.

CONCLUSION

The study showed the EA during HBO has greater effect in TBI patients than HBO or EA alone. Using the same therapy pressure, the $TcPO_2$ and GCS level measured in the EA during HBO groups was significantly higher than that of the other two groups. Thus, yielding a better therapeutic effect without jeopardizing patient safety. Using the same $TcPO_2$ (PaO_2) level, EA during HBO enabled a decrease in therapy pressure. Thus, this approach was very beneficial for TBI patients with a low tolerance for HBO such as children, older and the patients who suffer from contraindications for HBO therapy (e.g., severe upper respiratory tract infections, severe emphysema, bronchiectasis, severenasosinusitis) may need to undergo HBO treatment in cases of emergency. The results extend the range of HBO therapy and reduce the associated risks. Overall, the EA during HBO Method of HBO therapy demonstrated improvements in efficacy, safety and tolerability and should therefore be beneficial in a clinical context.

ACKNOWLEDGEMENT

Researchers wish to thank the Liu Chang, Wang Fei and 401st Hospital of the the PLA for technical assistance.

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