Dual effect of HBO on cerebral infarction in MCAO rats

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Received 5 May 2000; accepted in final form 1 November 2000

Badr, A. E., W. Yin, G. Mychaskiw, and J. H. Zhang. Dual effect of HBO on cerebral infarction in MCAO rats. Am J Physiol Regulatory Integrative Comp Physiol 280: R766–R770, 2001.—Various reports in the literature have shown that hyperbaric oxygen (HBO) reduces cerebral infarction both in animals and humans. After the initial ischemic insult, however, initiating HBO treatment at different intervals has yielded conflicting results. The present study was undertaken to determine the optimal therapeutic window in which to start HBO treatment for cerebral infarction after transient focal ischemia. In this study, the operator occluded the middle cerebral artery (MCA) of anesthetized rats by introducing a blunted nylon filament into the proximal MCA from the dissected external carotid artery. When the operator removed the filament after 2 h, focal ischemia and reperfusion occurred. The operator then placed the rat in the HBO chamber and administered 3 atm absolute HBO for 1 h according to the protocol. The rat was killed 24 h after reperfusion, and the percentage of infarction (infarct ratio) was calculated by dividing the infarction area by the total area of the ipsilateral hemisphere. The results showed that the percentage of infarcted area decreased significantly (P < 0.05) both in the 3- (7.59%) and 6-h (5.35%) HBO-treatment groups compared with the control (no treatment) group (11.34%). However, the percentage of infarcted area increased significantly (P < 0.01 and P < 0.05, respectively) both in the 12- (23%) and 23-h (20%) treatment groups. The results of this study suggest that applying HBO within 6 h of ischemia-reperfusion injury could benefit the patient but that applying HBO 12 h or more after injury could harm the patient.

Methods

The Animal and Ethics Review Committee at the University of Mississippi Medical Center evaluated and approved the protocol used in this study.

Experimental groups. Forty-eight rats, each weighing 325–375 g, were divided into six treatment groups each consisting of eight subjects. The operator in this study performed the following procedures for each respective treatment group: 1) sham operation, exposure of the left common carotid artery and left external carotid artery followed by neither MCAO, reperfusion, nor HBO treatment; 2) MCAO for 2 h followed by reperfusion for 24 h; 3) MCAO for 2 h followed by reperfusion for 24 h and then HBO treatment applied at 3 h after reperfusion; 4) MCAO for 2 h followed by reperfusion for 24 h and then HBO treatment applied at 6 h after reperfusion; 5) MCAO for 2 h followed by reperfusion for 24 h and then HBO treatment applied at 12 h after reperfusion; 6) MCAO for 2 h followed by reperfusion for 24 h and then HBO treatment applied at 23 h after reperfusion.

MCAO model and HBO treatment. Forty-eight male Sprague-Dawley rats (assigned by study protocol to MCAO) underwent the procedure described by Longa et al. (10a). Accordingly, the operator anesthetized the rats with ketamine and xylazine and exposed the left common carotid artery. Then, the external carotid artery and its branches were isolated and coagulated. A 3–0 nylon suture with a
blunted tip was inserted into the internal carotid artery through the external carotid artery stump and advanced to the anterior cerebral artery to occlude the middle cerebral artery (MCA). After occluding the MCA for 2 h, the operator carefully removed the suture to restore blood flow and then sutured the skin and allowed the rat to wake up. To complete the surgery, the operator applied 0.25% micaine locally to the wound and allowed the rat to recover.

After the treated rats were reperfused, they were placed in an HBO chamber (3 ATA for 1 h) at 3, 6, 12, and 23 h according to the protocol schedule for their group. When the HBO chamber had attained the desired pressure, the flow of oxygen was reduced to maintain constant pressure while allowing air exchange in and out of the chamber. This constant exchange was aided by a tray of calcium carbonate crystals placed inside the chamber to reduce CO₂ accumulation in the chamber environment; thus the oxygen level was maintained at or >98%, and the CO₂ level was maintained at or <0.03%. After HBO therapy, the operator returned the rat to its cage until death. The operator used a rectal probe to monitor the rat’s body temperature; during ischemia and postoperative recovery, body temperature was maintained at 37 ± 0.5°C. Death consisted of decapitation 24 h after the initial induced ischemia. After the rat’s death, the operator removed its brain.

Observing and evaluating the treated rats for neurological deficit. An experimenter, unaware of the treatments, tested the animals for neurological deficits after 24 h of reperfusion. Menzies et al. (11) developed the method that tests both motor and behavioral deficits on a cumulative scale from 0 to 4. This examination was used to evaluate ischemic injury: 0, no visible neurological deficits; 1, forelimb flexion; 2, contralateral forelimb grips weakly (the operator places the animal on an absorbent pad and gently pulls the tail); 3, circling to the paretic side only when pulled by the tail (the animal was allowed to move about freely on the absorbent pad); and 4, spontaneous circling.

Evaluation of infarcted area. After death had occurred, the coronal sections of the brain (2 mm thick) were cut and immersed in a 2% solution of 2,3,7-triphenyltetrazolium chloride. The stained slices were then fixed by immersion in phosphate-buffered 4% paraformaldehyde. The infarcted area and hemispheric area of each section were traced and measured using an image-analysis system (a Macintosh computer accessing the public domain National Institutes of Health Image program, written by Wayne Rasband and available from the internet). The percentage of infarction (infarct ratio) was calculated by dividing the infarcted area by the total area of the ipsilateral hemisphere (19).

Statistical analysis. Data were represented as the means ± SD. Statistical differences between the control (no HBO treatment) and the other groups were compared by using the one-way ANOVA and then Scheffe’s F test if a significant difference was found; a P value <0.05 was considered statistically significant.

RESULTS

The study operator performed a pilot study to determine the MCAO model used to conduct this study. The surgical method and the size of the suture and its blunted tips were tested in different animals until a consistent neurological deficit (evaluated 24 h after reperfusion) was obtained. The rats used for this pilot study were not included among the 48 rats described by the above protocol. No neurological deficit was observed in the sham-operated rats.

Figure 1 presents the typical results of the effect of MCA occlusion on infarction size from each of the groups: the sham-operated (no MCAO), MCAO-no HBO treatment, and HBO at 3, 6, 12, and 23 h after reperfusion. The coronal sections were obtained by cutting the brain at a distance of 2, 4, 6, 8, and 10 mm from the rostral extremity of the frontal cortex. The white-colored areas represent the infarction regions in these sections.

Figure 2 shows the results of the neurological deficit score in each group. Both the 3- and 6-h treatment groups exhibited a significantly improved neurological function (lower score) compared with the no-treatment group. In contrast, the neurological deficit was increased after HBO treatment at 12 and 23 h after reperfusion.

Figure 3 shows the percentage of infarction area in rats that underwent MCAO and reperfusion and MCAO/reperfusion + HBO therapy at 3, 6, 12, and 23 h. No cerebral infarction was observed in the sham group. In the MCAO and reperfusion group (untreated group), severe cerebral infarction was observed in all rats and the infarct ratio was 11.34%. The infarct ratio was significantly decreased in rats treated with HBO at 3 (7.59%, P < 0.05 vs. untreated group) and 6 h (5.35%, P < 0.05 vs. untreated group) after reperfusion. However, the infarct ratio was significantly increased in rats treated with HBO at 12 (23%, P < 0.01 vs. untreated group) and 23 h after reperfusion (20%, P < 0.05 vs. untreated group).

DISCUSSION

The primary finding of this study is that the effect of HBO on the cerebral infarction is dual in an MCAO and reperfusion rat model. This finding suggests the existence of an optimal therapeutic window in which to initiate HBO therapy in this rat model of cerebral ischemia: early therapeutic intervention using HBO within 6 h reduces infarction. Applying HBO therapy late (after 12 h, for example) aggravates cerebral infarction; thus caution needs to be a consideration of HBO therapy in such cases.

Because current stroke treatment is unsatisfactory, many physicians seek novel treatment modalities. Because local anoxia and energy failures occur at the cellular level in ischemia, increasing the oxygen delivery to the tissues might prolong functional activity during severe ischemia (14). Animal studies and clinical experiences over the last two decades have produced a set of applications for which HBO therapy might appear beneficial (1). However, most of these studies focused on forebrain ischemic or global ischemic models that might have impeded the investigation of therapeutic HBO applications. In designing the model for their study, these investigators chose to observe and evaluate the parameters of survival time and survival rate to determine the therapeutic effect of HBO (10). Moreover, neuronal injury in the forebrain
after brief global ischemia is a selective phenomenon that sometimes fully demonstrates only after observation for several days (19). By contrast, neuronal death, which follows an intense focal ischemic challenge in an area at risk for infarction, is evident within 12–24 h (19). The ischemic events characterizing neuronal death mimic more closely the clinical condition of ischemic stroke (19). Considering these conditions, we used an experimental MCAO and reperfusion model that represents a likely clinical problem: incomplete, focal ischemia with the potential for reperfusion. This model also mimics clinical disorders, such as large-vessel thrombosis, that lead to infarction, which is surrounded by ischemic penumbra. Theoretically, this model is an ideal candidate for therapeutic HBO (8).

Mainly, two patterns of cerebral damage characterize focal ischemia: focal damage and penumbral damage. Complete flow cessation causes the greatest damage at the focus. The penumbral tissues surrounding an ischemic focus sustain less severe damage because the collateral vessels supplying the penumbral area yield a residual perfusion. Oxygenation is the most critical function of blood flow; suddenly reduced oxygenation is an inevitable consequence of severe ischemia. If therapeutic intervention does not occur in a timely manner, penumbral zones could eventually lose their ability to maintain ionic homeostasis, thereby become subsumed into the focal area, and thus increasing the total ischemic brain damage. Through the administration of pharmacological agents, many investigators aim therapeutic stroke research at preventing the penumbral recruitment into the ischemic focus (6). Pharmacological intervention might either improve blood flow (i.e., oxygen supply) or protect against neuronal death. HBO treatment is considered for cerebral ischemia, because it might salvage the still viable, though nonfunctioning, tissues surrounding the infarcted area (14). Mink and Dutka. (12) reported that HBO can improve tissue oxygen delivery (especially to areas of diminished blood flow), can enhance neuronal viability, and can reduce brain edema. By raising the tissue $P_{O_2}$, HBO can trigger a mechanism controlling cellular and vascular repair. HBO therapy applied after radiation injury has demonstrated increased tis-
sue oxygen concentration, thereby stimulating angiogenesis and establishing a new capillary blood supply (5). HBO therapy salvages the still viable, though non-functioning, tissue surrounding the infarcted area presumably by allowing time for collateral circulation to develop. This collateralization forms the basis for the conclusion that HBO might benefit victims of cerebral ischemia. Our results showed that HBO therapy, when applied at an earlier stage after reperfusion (at 3 and 6 h), decreased the infarction area in the rat MCAO and reperfusion model. Our results agree with the report of Roos et al. (16) who state that HBO therapy in the rat MCAO model without reperfusion is beneficial and agrees with the report of Krakovsky et al. (10) about other animal models of cerebral ischemia.

Despite the beneficial effects resulting from HBO therapy applied at an earlier stage (i.e., at 3 and 6 h), our results also show that HBO therapy applied at a later stage (i.e., 12 and 23 h) can yield less beneficial effects in this rat MCAO and reperfusion model. When applied at a later stage, such as 12 or 23 h after reperfusion, HBO treatment increased the infarcted cerebral area. Although the exact mechanism remains unclear, several possibilities account for the harmful effect of applying HBO therapy later. Deleterious mechanisms involved in focal ischemic injury persist throughout the postischemic period. An unresolved controversy is whether these mechanisms are 1) triggered during ischemia and persist into the postischemic period, 2) triggered during reflow (reperfusion injury), or 3) both 1 and 2 foregoing. Canevari et al. (2) reported that in a rat MCAO model (2 h occlusion), recirculation for 1–2 h temporarily restored the bioenergetic state and mitochondrial function but that secondary deterioration occurred after 4 h. Gido et al. (7) stated that delayed cell membrane dysfunction, as reflected in a rise in K, occurs ~6 h after reperfusion.

Despite the suggestion that the generation of free radicals during reperfusion might cause secondary damage, it remains to be determined whether HBO applied at an early stage might prevent the generation of free radicals or the damage caused by free radicals and whether HBO application at a later stage enhances the harmful effect of free radicals.

Our finding differs from the findings of other studies that demonstrate the failure of HBO to exert a beneficial effect. These differences might be related to the experimental protocols of other investigations that prescribe prolonged HBO treatment. Long-term use of HBO results in adverse effects due to the onset of oxygen toxicity as manifested by induction of lipid peroxidation and seizures (14). Long-term HBO therapy might conflict with parameters such as the hematocrit level that, in turn, might influence viscosity (14). A previous study showed that the exposure of rats to 4 ATA of oxygen for 90 min was associated with an increased level of lipid peroxidation product (malondialdehyde) and altered the enzymatic antioxidation (glutathione peroxidase) in the brain (15). Chavko et al. (4) stated that some studies used 100% O2 at 5 ATA, which induced seizures. The Committee of the Undersea and Hyperbaric Medical Society recommends that a treatment pressure only from 2.4 to 3.0 ATA should be used at the lowest effective pressure to avoid O2 convulsions (3).

Perspectives

In conclusion, a dual effect of HBO on the cerebral infarction in MCAO rats was observed in this study, indicating that the optimal therapeutic window for HBO treatment should be restricted to <6 h after reperfusion. Further study is needed to clarify whether
HBO treatment provides long-term functional neuroprotection. Meanwhile, the mechanisms of HBO-induced neuroprotection remain unclear. In diseases other than cerebral ischemia, molecular events such as gene expression change occur after HBO treatment. This new direction warrants further investigation, because studies at the molecular level might not only assist in clarifying the mechanism of HBO, but also lead to identifying genes that could be neuroprotective. In this regard, an understanding of how HBO reduces brain damage after MCAO and reperfusion could be of tremendous value, because such understanding might lead to a more selective and effective therapy.

This work was partially supported by a grant-in-aid from the American Heart Association and by the Bugher Foundation Award for the investigation of stroke to J. H. Zhang.

REFERENCES