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HYPERBARIC OXYGEN TREATMENT ATTENUATED THE DECREASE IN REGIONAL GLUCOSE METABOLISM OF RATS SUBJECTED TO FOCAL CEREBRAL ISCHEMIA: A HIGH RESOLUTION POSITRON EMISSION TOMOGRAPHY STUDY

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Abstract—Cerebral hypoxia may be the main component of cell damage caused by ischemia. Previous studies demonstrated a neuroprotective effect of early hyperbaric oxygen (HBO) treatment in various animal models of focal cerebral ischemia. Neuropathologic study showed that exposure of HBO may prevent cell death in ischemic cortex. In the present study, we aimed to assess cellular function of ischemic rat brain after HBO treatment by means of a high-resolution positron emission tomography scanner (microPET) used specifically for small animal imaging. The male Sprague–Dawley rats were subjected to permanent middle cerebral artery occlusion (MCAO), with the regional cerebral blood flow monitored *in vivo* by laser Doppler flowmetry. One hour after ischemia, HBO therapy (3 atm absolute, 1 h) was initiated. Local cerebral glucose utilization in the ischemic area was measured before, 1 h and 3 h after ischemia, with 2-[^{18}F]-fluoro-2-deoxy-d-glucose (FDG) as a tracer. Neurological deficits and infarct volumes were assessed at 24 h after ischemia. Our study showed that early HBO therapy significantly reduced infarct volume of brain 24 h after ischemia. Moreover, glucose utilization in the ischemic area underwent a severe decrease during 1–3 h after MCAO, while the early HBO treatment significantly attenuated the decrease in cerebral metabolic rate of glucose in the ischemic core of the cortex compared with controls. We report for the first time the application of microPET to quantify the rates of glucose metabolism in the ischemic core of rats exposed to HBO. Our results suggest that the early exposure of HBO can partially reverse the downward trend for glucose utilization in the ischemic core, which might contribute to the reported beneficial effects of early HBO therapy on permanent cerebral ischemia.

Key words: hyperbaric oxygen, brain ischemia, positron emission tomography, glucose utilization.

As a highly energy-consuming organ, the brain is vulnerable to glucose and oxygen deprivation. During cerebral ischemia, the reduction in the supply of oxygen and glucose to the brain leads to mitochondrial oxidative phosphorylation reduction and a rapid loss of high-energy phosphates such as ATP. It subsequently causes irreversible neuronal dysfunction in a short time (Taylor et al., 1985). Therefore, the metabolism of glucose and mitochondrial oxygen is a centrally important cellular function that apparently shows long-term alterations following focal cerebral ischemia (Sims and Anderson, 2002).

By increasing the oxygen content of blood and improving tissue oxygenation, hyperbaric oxygen (HBO) treatment has been implicated as an attractive procedure for use in cerebral ischemia. Multiple studies demonstrated neuroprotective effects of early HBO treatment in various animal models of focal cerebral ischemia (Lou et al., 2004; Schabitz et al., 2004; Sunami et al., 2000). HBO therapy was shown to increase tissue oxygen delivery, regulate post-ischemia metabolism, reduce brain edema, prevent apoptosis and enhance neuronal viability in ischemic rats (Badr et al., 2001; Sunami et al., 2000; Veltkamp et al., 2005; Yang et al., 2002; Yin et al., 2003). However, most of these results come from the neuropathologic analysis, without *in vivo* evaluation of neuronal function.

Positron emission tomography (PET) is a noninvasive imaging technique that allows quantitative *in vivo* determinations of the rates of various physiologic and biochemical processes, with minimal invasiveness when doing so. Recently, improvement in scanner resolution has allowed PET to become a potential method to monitor cerebral metabolic patterns in rat brain using small animal positron emission tomography (microPET) (Cherry, 2004; Chatziio-

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Abbreviations: ATA, atm absolute; FDG, 2-[^{18}F]-fluoro-2-deoxy-D-glucose; GLUT, glucose transporters; HBO, hyperbaric oxygen or hyperbaric oxygenation; MCAO, middle cerebral artery occlusion; microPET, high-resolution positron emission tomography; PET, positron emission tomography; rCBF, regional cerebral blood flow; rCMRgl, regional cerebral metabolic rate for glucose; ROIs, regions of interest; SD, Sprague–Dawley; TTC, 2,3,5-triphenyltetrazolium chloride.

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annou et al., 2001; Tai et al., 2003). These scanners produce high quality images that provide the researcher with information on relative tracer uptake in rats. Moreover, microPET can also be used in the early stage of acute brain lesions, which cannot be accomplished in the clinical PET studies because tracer kinetics is unwarranted and image acquisition is difficult in the early phase of acute ischemic stroke (Cherry and Gambhir, 2001).

The tracer 2-[^{18}F]-fluoro-2-deoxy-D-glucose (FDG) is the well-known radiotracer that frequently has been used as a marker of metabolic activity for glucose. The level of glucose utilization correlates with the degree of neuronal activity (Chugani et al., 1991; Bruehl and Witte, 1995). Previous studies, with a microdialysis system, found that early HBO treatment decreased glucose concentration in striatal extracellular fluid after focal cerebral ischemia (Badr et al., 2001). We thus deemed it worthy to investigate whether HBO treatment enhances the glucose utilization, which might be involved in the protective effect of HBO in cerebral ischemia. Therefore, in the present study, a high-resolution microPET scanner that employs novel detector technology and that has been designed specifically for small animal imaging was used to investigate the rate of glucose utilization during middle cerebral artery occlusion (MCAO) in rats. The effect of HBO on the glucose utilization was measured in the ischemic cortex at different duration after ischemia.

EXPERIMENTAL PROCEDURES

This study was approved by the Animal Research Committee of Zhejiang University, School of Medicine. The study was carried out using male Sprague–Dawley (SD) rats weighing 200 g. The experimental procedure was approved by the Animal Research Committee of Zhejiang University, School of Medicine, and was conducted in accordance with the guidelines of the U.S. National Institutes of Health on the Care and Use of Laboratory Animals. Every effort was made to minimize the number of animals used in these studies and their suffering. After an overnight fast, chloral hydrate (400 mg/kg) injected intraperitoneally was used as anesthetic for all surgical procedures.

Animals were randomly assigned to the following groups: group A (baseline group): FDG injection and scans were performed on two rats 1 week before operation; group B (HBO without ischemia group): HBO initiated 1 h and subsequent FDG injection 3 h after sham-operation ($n=2$); group C (ischemia 1 h group): FDG injection starting 1 h after ischemia ($n=3$); group D (ischemia 3 h group, control group): FDG injection starting 3 h after ischemia ($n=10$); group E (HBO-treated group): HBO initiated 1 h after ischemia and subsequent FDG injection starting 3 h after ischemia ($n=10$).

Permanent MCAO

Permanent focal cerebral ischemia was produced by intraluminal suture occlusion of the right MCAO using a 4-0 silicone-coated nylon filament. Regional cerebral blood flow (rCBF) was continuously monitored at one point (1 mm posterior to the bregma, 5 mm from the midline) on the surface of right hemisphere in the supply territory of the MCA before, during MCAO and immediately after HBO treatment by laser-Doppler-flowmetry (Periflux system 5000; Perimed, Stockholm, Sweden), as we used previously (Lou et al., 2006). Abrupt reduction in rCBF by approximately 70–80% indicated a successful occlusion of the MCA. Rats in which the ipsilateral blood flow during ischemia was not reduced to less than 30% of baseline during the first 30 min of occlusion, or in which a premature increase in the ipsilateral blood flow was recorded, were excluded from the experiments. Body temperature was maintained at 37 °C with a heating pad. Physiological parameters (rectal temperature, arterial pH, PCO_2, PO_2, glucose, potassium, sodium, and chloride) were taken and analyzed in each group throughout the studies (Roche OMNI C; Roche Diagnostics GmbH, Mannheim, Germany). Blood samples were taken at baseline, 5 min after MCAO, and 1 min after HBO or control condition.

HBO therapy

HBO was performed in an experimental pressure chamber. HBO was administered at a pressure of 3 atm absolute (3 ATA) for 1 h with 100% oxygen, starting at 1 h after MCAO. Compression and decompression were achieved within 5 min. The control group received the same dose of anesthesia corresponding to time points of HBO.

FDG injections and microPET scans

FDG, with a specific activity of 500 Ci/mmol, was prepared in the Department of Nuclear Medicine. Rats were anesthetized and injected i.v. with 0.5 mCi of pyrogen-free FDG into the tail vein, after which they were returned to their home cage in a room with minimal ambient noise for the duration of the uptake period. Following FDG uptake, the animals were placed in the microPET scanner, which consists of a 15 cm diameter ring of 96 position-sensitive γ-ray scintillation detectors with an intrinsic resolution <1.8 mm. Three-dimensional volumetric images were reconstructed using a maximum a posteriori probability algorithm and the regional glucose metabolic rate was determined. Image resolution was 1.5 mm in the maximum a posteriori probability reconstructions and the transaxial image planes were separated by 1.21 mm. To determine appropriate times for data acquisition, quantitative dynamic scans were performed on six adult rats at 60, 120, 180 min following injection respectively. No differences were found for cortical metabolic rates at the different times following injection. Therefore, for the relative quantitative studies presented here, we used images summed and averaged at 120 min post-FDG injection. Sufficient counts were collected on each scanner to ensure that the data were limited by the resolution of the imaging device and not by statistical considerations.

Evaluation of neurological deficits

The neurological status of each rat was evaluated 24 h after MCAO by a blinded observer. The Garcia neurological grading systems were used to assess the effects of HBO (Garcia et al., 1995).

Measurement of infarct volume

Twenty-four hours after ischemia, histologic staining was performed using 2,3,5-triphenyltetrazolium chloride (TTC). Serial 1.2-mm thick coronal sections were obtained in parallel with the PET planes according to the stereotaxic rat-brain atlas (Paxinos and Watson, 1998). The stained sections were then photographed and infarct volumes were determined using ImageJ software (http://rsb.info.nih.gov/ij/). To compensate for the effects of brain edema, the corrected infarct volume was calculated as described in detail by Schabitz et al. (2000).

Calculation of regional cerebral metabolic rate for glucose (rCMRgl)

The representative coronal slices from FDG-PET images and the TTC-stained pictures from the same rat were manually compared.

Analysis of scans was accomplished by drawing the regions of interest (ROIs) around the area of hypometabolism in right parietal cortex on the microPET images, which was within the ischemic core in the corresponding TTC-stained slice. Two contiguous coronal slices containing the selected region on the microPET images were analyzed, and the average density for the same-sized ROIs from two slices was calculated. The metabolic activity of the left cortex was also evaluated. Results were expressed as percentage hypometabolism obtained by the formula: average signal intensity right/average signal intensity left×100, as described by Kornblum et al. (2000). Therefore, values between 1 and 100 denote depression. To verify the reproducibility of the ROIs placement, two different readers separately drew the ROIs on the same regions of each animal and the results were compared.

Statistical analyses

Student's *t*-test was used for comparison of physiological parameter, percentage hypometabolism of glucose, neurological score and infarct volume between control and HBO-treated group. Comparison of percentage hypometabolism of glucose between 1 h and 3 h control group was also made by Student's *t*-test. Linear regression analysis was performed to correlate the percentage hypometabolism of glucose by 3 h with TTC-defined cortical infarct volumes in the same control rats. A probability value <0.05 was considered significant. Data are expressed as means±S.E.M.

RESULTS

Physiological parameters

With the exception of post-HBO pO_2 levels in the HBO-treated group (117.1±5.00 mm Hg versus 85.2±3.48 mm Hg [Control], $P<0.01$, $n=10$), arterial pH, pCO_2, potassium, sodium, chloride and blood glucose were not significantly different between the groups (Table 1).

Table 1. rCBF, arterial blood oxygen, carbon dioxide, and pH values and glucose, sodium, potassium, and chloride concentrations in control and HBO-treated rats throughout the study

Parameter	Control ($n=10$)	HBO ($n=10$)
Baseline		
pH	7.30±0.03	7.30±0.01
pCO_2, mm Hg	63.0±4.79	63.4±2.26
pO_2, mm Hg	83.3±4.85	84.7±1.03
Glucose, mg/dl	136.8±6.52	136.5±7.85
K^+, mmol/l	4.34±0.18	4.68±0.13
Na^+, mmol/l	133.1±1.54	133.5±0.85
Cl^-, mmol/l	95.0±2.58	96.0±1.58
5 Min after ischemia		
rCBF, % of baseline	19.1±2.45	19.2±2.47
pH	7.30±0.02	7.30±0.01
pCO_2, mm Hg	63.8±3.52	63.7±3.30
pO_2, mm Hg	83.9±4.28	84.3±1.46
Glucose, mg/dl	134.5±9.20	133.8±9.05
K^+, mmol/l	4.38±0.16	4.86±0.06
Na^+, mmol/l	131.1±0.90	132.3±0.72
Cl^-, mmol/l	94.2±1.04	97.0±1.53
1 Min after control condition or HBO		
rCBF, % of baseline	20.3±2.80	19.7±2.44
pH	7.30±0.03	7.31±0.02
pCO_2, mm Hg	60.3±1.08	60.3±1.68
pO_2, mm Hg	85.2±3.48	117.1±5.00**
Glucose, mg/dl	133.5±11.3	140.2±10.2
K^+, mmol/l	4.71±0.33	4.62±0.19
Na^+, mmol/l	132.3±1.16	132.3±1.33
Cl^-, mmol/l	96.8±1.85	97.9±1.85

Post-HBO pO_2-levels in the HBO-group were significantly higher compared to baseline values as well as values in the control group at the same time (**$P<0.01$).

rCBF

An immediate decrease of the blood flow displayed the correct position of the filament at the origin of the MCA. The low blood flow was maintained at approximately 20% of the pre-ischemic baseline level during the monitoring after permanent MCAO. No difference in rCBF was seen between the groups. HBO treatment did not significantly change the regional blood flow (Table 1).

Neurological evaluation

At baseline, the neurological status was equal between the groups. The total neurological score was increased in HBO-treated group but failed to reach statistical significance (11.1±0.47 [Control] versus 12.2±0.30 [HBO-treated], $P=0.077$, $n=10$). However, when we separately analyzed the six functions of the Garcia grading system, the significant improvement in symmetry of the movement of four limbs and forepaw outstretching was observed in the HBO-treated rats 24 h after ischemic onset (Fig. 1b).

Infarct volumetry

Tissue sections stained with TTC showed a reproducible infarct zone involving the frontoparietal neocortex, caudate putamen, and globus pallidus in control animals. A significant reduction of the infarct volume was found when HBO initiated 1 h after MCAO (Fig. 2). The total infarct volume was reduced by 25% (202±12 mm^3 [Control] versus 153±18 mm^3 [HBO-treated], $P<0.05$, $n=10$) and cortical infarct volume was reduced by 32% (129±8 mm^3 [Control] versus 90±15 mm^3 [HBO-treated], $P<0.05$, $n=10$).

rCMRgl

FDG-microPET images were of high quality and allowed delineation between cortical and subcortical structures. Baseline quantitative microPET studies did not show metabolic asymmetry in any structure. Representative FDG-microPET images are presented in Fig. 3. There was no metabolic asymmetry in the right and left hemispheres of rat receiving HBO after sham-operation (Fig. 3A). By 1 h after MCAO, the mild decrease was observed in the right parietal cortex (Fig. 3B), while a significant increase was evident in the border of ischemic area from the surrounding slice (the upper slice in Fig. 3B). By 3 h after MCAO, the hypometabolic region was enlarged distinctly and great decreases were observed in the right parietal cortex (Fig. 3C), while it was lessened significantly in the ischemic rats receiving HBO 1 h after MCAO (Fig. 3D), compared with controls. Quantitative analysis showed that metabolic activity of ischemic core in the parietal cortex underwent a

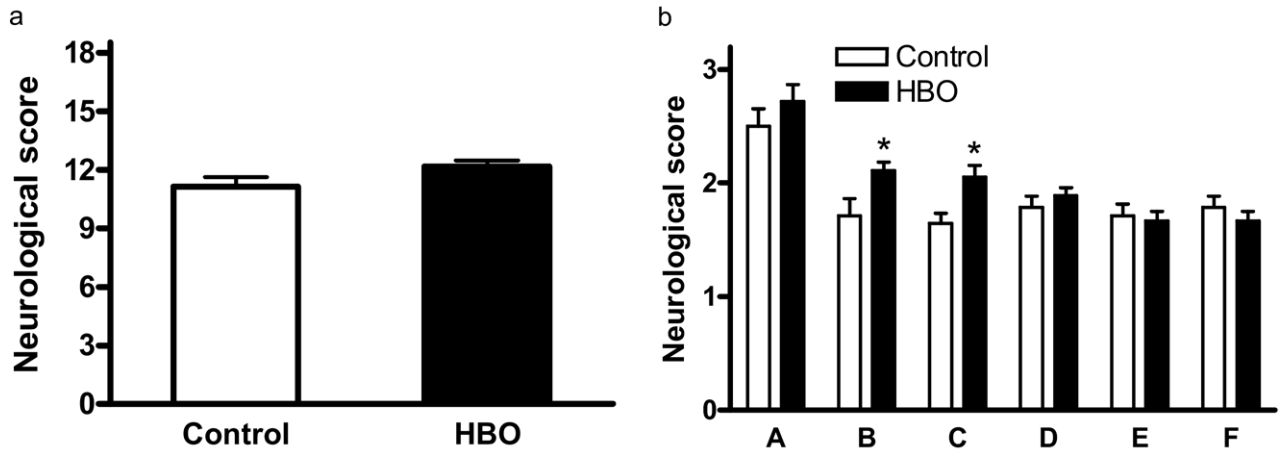

Fig. 1. Neurological scores in the rats assessed by the Garcia grading system at 24 h after ischemia. Rats with better neurologic outcomes received higher neurological scores. The total neurological score was increased in HBO-treated group but failed to reach statistical significance (a). However, the significant improvement in symmetry of the movement of four limbs (B) and forepaw outstretching (C) were observed in the HBO-treated rats (b), while there were no significant differences in spontaneous activity (A), climbing (D), body proprioception (E) and response to vibrissae touch (F). Neurological scores are expressed as mean±S.E.M., $n=10$, * $P<0.05$, statistical comparisons with the control group.

significant depression from 1 to 3 h after ischemia (Fig. 4). However, substantial increase of metabolic activity in the ischemic core was observed in the HBO-treated rats (68±5.5 [Control] versus 84±3.4 [HBO-treated], $P<0.05$, $n=10$). Regression analysis indicated the infarct volume of cortex at 24 h after ischemia was inversely related to the percentage hypometabolism of glucose in the cortical ischemic core at 3 h of MCAO ($r^2=0.728$, $P<0.05$, $n=10$).

DISCUSSION

In the present study, we investigated *in vivo* whether HBO treatment reduces infarct volume and whether early HBO treatment influences glucose utilization during focal cerebral ischemia. The major findings in the present study include: (1) HBO (3 ATA, 60 min) initiated 1 h after permanent MCAO reduced infarct volume. (2) The rate of glucose utilization in the ischemic core underwent a severe decrease during 1–3 h after MCAO. (3) Early HBO treatment significantly attenuated the decrease in the rate of glucose utilization in the ischemic core. Our results suggested that the maintenance of glucose utilization in the ischemic core by HBO might contribute to the neuroprotective effect of HBO treatment. To the best of our knowledge, we reported for the first time the application of microPET to quantify rates of glucose metabolism in the ischemic rats exposed to HBO therapy.

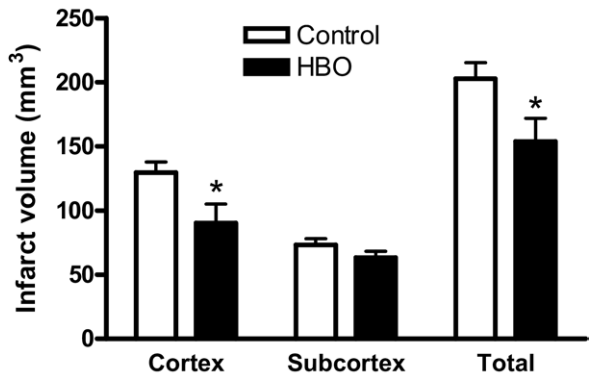

Fig. 2. Infarct volumetry assessed 24 h after MCAO. Total (hemispheric), cortical and subcortical infarct volumes are displayed separately. HBO treatment significantly reduced the cortical and total infarct volume. Data are presented as mean±S.E.M., $n=10$, * $P<0.05$ statistical comparisons with the control group.

HBO has proved highly effective in reducing lesion size and improving neurologic outcome in conditions like stroke (Gunther et al., 2005; Yang et al., 2002; Schabitz et al., 2004; Sunami et al., 2000). Our previous studies suggested that the therapeutic window for the single use of HBO may be approximately 6 h in transient MCAO, but no neuroprotective effect was found even in HBO administered 3 h after permanent MCAO (Lou et al., 2004). The importance of the therapeutic window concept may explain the failure of some previous HBO studies where therapy was considerably delayed (Anderson et al., 1991; Roos et al., 1998). We doubted that HBO administered 3 h after permanent MCAO might also be delayed, thus, the effect of HBO treatment used 1 h after permanent MCAO was further investigated in the present study. Interestingly, we found that the infarct volume was significantly reduced by 25% when HBO initiated 1 h after MCAO. It was reported that 2–4 h of focal ischemia caused as great a degree of infarction as permanent focal ischemia in rats (Kaplan et al., 1991; Memezawa et al., 1992), suggesting the importance of the oxygen and/or glucose supply during the first 2–4 h of focal ischemia. Therefore, an earlier therapeutic window of HBO treatment is needed for cerebral ischemia without reperfusion than with reperfusion. Additionally, HBO treatment improved only the recovery of motor functions at 24 h after ischemia, including symmetry of movement of four limbs and forepaw outstretching, while the overall improvement in the neurologic deficits failed to reach statistical significance. This finding stresses the im-

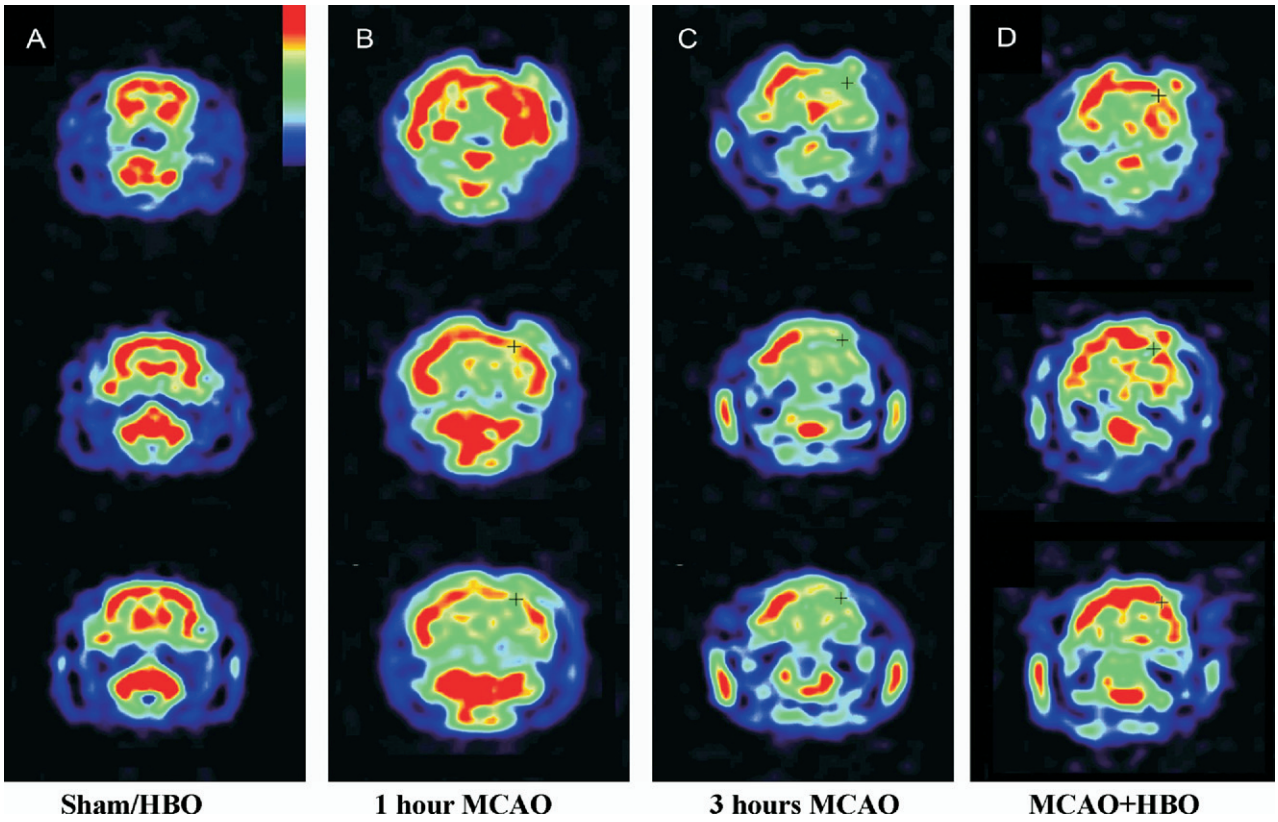

Fig. 3. Effects of HBO treatment on metabolic activity of glucose imaged on the microPET scanner. Images were taken from the same scan and reconstructed using the maximum a posteriori probability algorithm. There was no metabolic asymmetry in the right and left hemisphere of rat receiving HBO after sham-operation (A). The rCBF was decreased in the right hemisphere detected by laser-Doppler-flowmetry in the supply territory of the MCA (see cross in B, C, D). By 1 h after MCAO, the mild decrease of metabolic activity of glucose was observed in the right parietal cortex (B), while a significant increase was also evident in the border of ischemic area. By 3 h after MCAO, the hypometabolic region was enlarged distinctly in the control group (C), while it was lessened significantly in the ischemic rats receiving HBO 1 h after MCAO (D).

portance of a separate evaluation of motor and sensory functions in the assessment of the functional benefit of potential neuroprotective agents. HBO may improve motor dysfunction after ischemic insult, which represents the major focus in stroke rehabilitation.

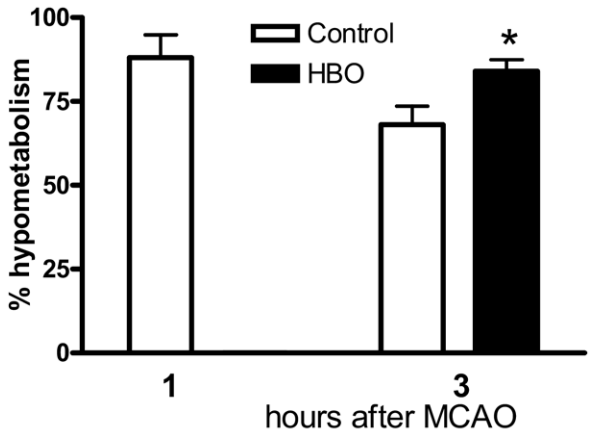

Fig. 4. Relative quantitation of the metabolic activity of ischemic core in the cortex following MCAO. Results were expressed as percentage hypometabolism obtained by the formula: average signal intensity right/average signal intensity left×100. Metabolic activity of ischemic core in the parietal cortex underwent a significant depression from 1 to 3 h after ischemia, while substantial increase was observed in the HBO-treated rats. Data are presented as mean±S.E.M., * $P<0.05$ statistical comparisons with the control group by 3 h after MCAO ($n=10$).

Our studies also showed the major changes in cellular handling of glucose in the post-ischemic brain. In fact, similar changes were also reported previously by the use of [^{14}C]deoxyglucose autoradiographic method (Belayev et al., 1997). The mild decrease in glucose utilization at 1 h of ischemia may be explained by the rapid increase of anaerobic glycolysis of cerebral glucose. It was shown that the activity of phosphofructokinase, the principal rate-limiting enzyme of glycolysis, was stimulated when ATP concentration was low (Erecinska, 1989). On the other hand, the excessive release of glutamate under oxygen deprivation also directly contributes to the heterogeneous acceleration of glucose metabolism (Sasaki et al., 2005). However, the sustaining imbalance between the reduced energy supply and normal cellular energy demand triggers various metabolic cascades, which lead to irreversible injury and cell death (Lipton, 1999; Jonas et al., 1999). Thus, glucose utilization finally fell to ~60% normal by 3 h, suggesting significant compromise of cell function at this point. This is consistent with the previous result which showed massive pan-cellular death in the core within 3 h in SD rats (Osborne et al., 1987). In contrast, the increased

rCMRgl was clearly observed in the ischemic core after being exposed to HBO treatment by 3 h of ischemia in the present study. Such reversal of the downward trend for glucose utilization distinctly indicates that cell function in the ischemic core was partially preserved by HBO at that time point. Moreover, we found that the cortical infarct volume at 24 h after ischemia was inversely related to the percentage hypometabolism of glucose at 3 h of MCAO in the same rat. It is likely that the compromise of cell function in the ischemic core by 3 h of ischemia may predetermine the subsequent ischemic injury. Thus, our results suggested that the preserved glucose utilization by HBO might prevent the progression of tissue infarction, via the maintenance of cell function in the ischemic core.

It is shown that a major determinant of the uptake of FDG is the activity of Na^+,K^+-ATPase, which represents ~50% of the total cerebral energy consumption. HBO treatment has been previously reported to preserve ischemia-induced significant decrease in the hippocampal Na^+,K^+-ATPase activity during global cerebral ischemia (Mrsic-Pelcic et al., 2004). Although this might account for the increased uptake of FDG, the precise details of the relationship between HBO treatment and improved glucose utilization remain obscure. Our data showed that the effect of HBO on glucose utilization did not result from the increase of blood glucose, indicating that changes in oxygen did not affect supply of glucose. With the use of a microdialysis system, Badr et al. (2001) found that glucose concentration in striatal extracellular fluid was increased after ischemia-reperfusion, while HBO treatment decreased glucose almost to the control (preocclusion) level. Actually, it is clear that FDG used in tracer concentrations is a valuable marker of glucose uptake and phosphorylation, since it is transported into cells through glucose transporters (GLUT) and phosphorylated by hexokinase to glucose 6-phosphate. Glucose enters the brain through 55-kDa GLUT-1 localized on endothelial cells of the blood–brain barrier, whereas GLUT-3 mediates this process in neurons (Lubec et al., 2000). Recently, it was demonstrated that HBO treatment promoted a transient increase in the expression of GLUT-1 and GLUT-3 after a neonatal hypoxic–ischemic insult (Calvert et al., 2006). The present results suggest that the transient upregulation of the expression of GLUTs by HBO treatment may contribute to the reversed uptake of FDG. Future work will focus on the interactions of HBO treatment and the expression of the GLUT family.

CONCLUSION

In conclusion, we observed that early exposure of HBO can partially reverse the downward trend for glucose utilization in the ischemic core of the cortex, which might contribute to the reduction of the infarct volume after permanent cerebral ischemia in rats. This finding has significant implications for our understanding of the neuroprotective effect of early HBO therapy. It is assumed that cell death observed in the infarct core is beyond the reach of therapeutics (Jonas et al., 1999), the present study provides a novel target for study to explore the cellular mechanism in the ischemic core.

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