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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.