Ethanol and Normobaric Oxygen

Novel Approach in Modulating Pyruvate Dehydrogenase Complex After Severe Transient and Permanent Ischemic Stroke

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Background and Purpose—Ischemic stroke induces metabolic disarray. A central regulatory site, pyruvate dehydrogenase complex (PDHC) sits at the cross-roads of 2 fundamental metabolic pathways: aerobic and anaerobic. In this study, we combined ethanol (EtOH) and normobaric oxygen (NBO) to develop a novel treatment to modulate PDHC and its regulatory proteins, namely pyruvate dehydrogenase phosphatase and pyruvate dehydrogenase kinase, leading to improved metabolism and reduced oxidative damage.

Methods—Sprague–Dawley rats were subjected to transient (2, 3, or 4 hours) middle cerebral artery occlusion followed by 3- or 24-hour reperfusion, or permanent (28 hours) middle cerebral artery occlusion without reperfusion. At 2 hours after the onset of ischemia, rats received either an intraperitoneal injection of saline, 1 dose of EtOH (1.5 g/kg) for 2- and 3-hour middle cerebral artery occlusion, 2 doses of EtOH (1.5 g/kg followed by 1.0 g/kg in 2 hours) in 4 hours or permanent middle cerebral artery occlusion, and EtOH+95% NBO (at 2 hours after the onset of ischemia for 6 hours) in permanent stroke. Infarct volumes and neurological deficits were examined. Oxidative metabolism and stress were determined by measuring ADP/ATP ratio and reactive oxygen species levels. Protein levels of PDHC, pyruvate dehydrogenase kinase, and pyruvate dehydrogenase phosphatase were assessed.

Results—EtOH induced dose-dependent neuroprotection in transient ischemia. Compared to EtOH or NBO alone, NBO+EtOH produced the best outcomes in permanent ischemia. These therapies improved brain oxidative metabolism by decreasing ADP/ATP ratios and reactive oxygen species levels, in association with significantly raised levels of PDHC and pyruvate dehydrogenase phosphatase, as well as decreased pyruvate dehydrogenase kinase.

Conclusions—Both EtOH and EtOH+NBO treatments conferred neuroprotection in severe stroke by affecting brain metabolism. The treatment may modulate the damaging cascade of metabolic events by bringing the PDHC activity back to normal metabolic levels. (Stroke. 2015;46:492-499. DOI: 10.1161/STROKEAHA.114.006994.)

Key Words: EtOH ■ ischemia–reperfusion injury ■ metabolism ■ pyruvate dehydrogenase complex ■ pyruvate dehydrogenase kinase ■ pyruvate dehydrogenase phosphatase ■ reactive oxygen species

Stroke is one of the most debilitating vascular diseases worldwide, which is keeping our healthcare costs as high as $38.6 billion each year.1 Systemic thrombolysis with intravenous tissue-type plasminogen activator and in situ clot retrieval remain the only reperfusion strategies approved by the Food and Drug Administration. However, after nearly 2 decades of research has failed to develop targeted therapies to address plasminogen activator’s contraindications, and most importantly, its narrow therapeutic time window. Furthermore, while a small portion of patients (17%) undergo spontaneous lysis by 6 to 8 hours,2 many patients experience permanent artery occlusion.1 In addition, even if recanalization is successful, outcome is often poor because of reperfusion injury.4

Although energy failure and oxidative stress with reactive oxygen species (ROS) generation after ischemia are well-documented pathophysiologies of neural injury, recent research has failed to develop targeted therapies to address...
these dysfunctions that may confer neuroprotection acutely after stroke. Immediately after brain stroke, a shift from aerobic to anaerobic metabolism occurs in ischemic tissue. Pyruvate dehydrogenase complex (PDHC), a key element in cellular metabolism, as well as its strategic regulators, is the link between aerobic and anaerobic energy metabolism. It is composed of multiple subunits that altogether convert pyruvate to acetyl coenzyme A to eventually make ATP needed for different cellular functions. PDHC’s vast size and strict regulation makes it sensitive to inactivation and downregulation in stroke. Two key regulators of PDHC are pyruvate dehydrogenase kinase (PDK) and pyruvate dehydrogenase phosphatase (PDP). Notably, PDK actively phosphorylates various sites on the E1α subunit of PDHC resulting in the inhibition of the entire enzyme complex. Conversely, PDP removes the phosphate group off the serine residues and thus activates the enzyme. Ultimately, PDHC, PDK, and PDP changes after stroke would provide important indexes to assess oxidative metabolism underlying ischemic injury after stroke.

Previous studies have demonstrated that EtOH decreases brain catabolism, raising its potential as a clinical neuroprotectant in ameliorating metabolic dysfunction after stroke. In rats with 2-hour middle cerebral artery occlusion (MCAO), we previously showed that a postschemia moderate but not a lower dose of EtOH reduced brain infarction and improved functional outcome in rats. Oxygen administration has been identified as a rational strategy for stroke therapy, and normobaric oxygen (NBO) is readily available, inexpensive and can be quickly applied. However, the time window for NBO is short, and its therapeutic effect is relatively low in both transient and permanent focal ischemia. Considering the brain metabolic depressive and protective effects of EtOH, and the beneficial effects of NBO on metabolic stress after stroke, as well as the fact that both agents can be easily delivered through the blood brain barrier into the ischemic brain, we aimed at determining the therapeutic effect of EtOH or EtOH+NBO in stroke by normalizing oxidative metabolism through various regulatory mediators of PDHC. Because an early reperfusion strategy may not be available for most patients, and because the proposed treatments can be effectively administered and delivered to ischemic regions through the collateral circulation that remains patent in patients with stroke, we used more clinically relevant stroke models with longer ischemia periods (4 versus 2 or 3 hours) or without reperfusion (permanent stroke).

Materials and Methods

Subjects
All experimental procedures were approved by the Institutional Animal Investigation Committee of Wayne State University in accordance with the National Institutes of Health guidelines for care and use of laboratory animals. A total of 176 adult, male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) were randomly divided into the following groups such as (1) a sham-operated group without MCAO (n=8), (2) 2-hour MCAO (n=8x2), (3) 3-hour MCAO (n=8x2), (4) 4-hour MCAO (n=8x9), and (5) permanent MCAO (28 hours; n=8x8). The 2- and 3-hour MCAO groups were randomly assigned to receive either saline (sham treatment) or an intraperitoneal injection of EtOH (1.5 g/kg) at 2 hours after the onset of ischemia. For the 4-hour MCAO, rats in total 9 groups were randomly assigned to receive 3 different treatments (saline, 1 dose [1.5 g/kg] or 2 doses [1.5 g/kg at 2 hours after the onset of MCAO followed by 1.0 g/kg in 2 hours], 95% NBO [at 2 hours after the onset of ischemia for 6 hours], or EtOH+95% NBO); and were processed for infarct volume and biochemical analyses, respectively, at 28 hours after ischemia. The mortality rate was low (<9%) and was about equal in each group. All data were analyzed in a blind manner. Animals were fed the night before.

Middle Cerebral Artery Occlusion
Rats were subjected to MCAO for either 2, 3, 4, or 28 hours using the intraluminal filament model. Reperfusion was achieved by the withdrawal of the filament at each corresponding time point as mentioned above. Blood Pco2 and Po2, and mean arterial pressure were monitored throughout the procedure. Heating lamps and pads were used to maintain rectal temperature at 36.5 to 37.5°C. See online-only Data Supplement for details.

EtOH Treatment
In transient ischemic stroke with 2-hour MCAO, the treatment was given immediately before reperfusion both to mimic actual scenarios encountered in clinical settings and to prevent reperfusion injury. In other MCAO groups, all ischemic rats received 1 EtOH dose (1.5 g/kg IP) at 2 hours after ischemia onset (the time point with a confirmed EtOH neuroprotective effect). Rats in the 4 hours and permanent MCAO groups received a second EtOH dose (1.0 g/kg) at 2 hours after the first dose.

NBO Treatment
To receive NBO treatment, rats at 2 hours after the onset of ischemia were placed in a sealed chamber (50x25x25 cm) full of 95% oxygen for 6 hours. To maintain oxygen concentration, an oxygen controller (PRO-OX110; Reming Bioinstruments Co, Redfield, NY) was used and the oxygen flow rate was 2 L/min. In addition, carbon dioxide was removed by placing a container of soda lime (Sigma) at the bottom of the chamber.

Combination Treatment With NBO and EtOH
At 2 hours after the onset of ischemia, animals that received IP injections of EtOH as described above were then placed into a sealed chamber full of 95% oxygen to undergo consecutive NBO treatment for 6 hours.

Neurological Deficit
The scoring system proposed by Belayev et al was used to examine the severity of deficit in rats before surgery, during MCAO after 24-hour reperfusion or 28-hour MCAO without reperfusion.

Cerebral Infarct Volume
Twenty-four hours after reperfusion or 28 hours after MCAO without reperfusion, brains were obtained from ischemic rats and cut into a 2-mm thick slices and treated with 2, 3, 5-triphenyltetrazolium chloride (Sigma) for staining. An indirect method for calculating infarct volume was used to minimize error caused by edema.

ADP/ATP Ratio
Brain metabolism was measured with the BioVision ApoSENSOR Assay Kit (Mountain View, CA), as described by us. See online-only Data Supplement.

ROS Production
ROS was detected as described previously by us. See online-only Data Supplement.
Protein Expression

Western blot was used to detect protein levels of PDHC, PDP, and PDK. Brain tissue samples containing frontoparietal cortex and dorsolateral striatum, the MCA supplied territories, were processed as described previously by us\textsuperscript{8,19} using primary antibody incubation (PDHC, 1:250; PDP1, 1:1000; PDP2, 1:1000; PDK, 1:1000; Santa Cruz Biotechnology, Dallas, TX) at 4°C. See online-only Data Supplement.

Statistical Analysis (SPSS Software, Version 17, SPSS Inc)

All the data were described as mean±SE. Differences among multiple groups were assessed using both the 1- and 2-way ANOVA with a significance level at \( P<0.05 \). Post hoc comparison between groups was detected using the least significant difference method.

Results

Physiological Parameters

There were no significant differences in blood pH and \( \text{PCO}_2 \) in each group. However, a significantly (\( P<0.05 \)) increased arterial blood \( \text{PO}_2 \) was found in the NBO-treated groups. Body and brain temperature remained at \( \approx 37°C \). See online-only Data Supplement.

Therapeutic Window of EtOH, NBO, and Combined Treatment

In ischemic rats with 2- or 3-hour MCAO, a single dose of EtOH (1.5 g/kg) significantly (\( P<0.01 \)) decreased infarct volumes as compared with no-treatment groups (Figure 1A). Only a mild but nonsignificant decrease in infarct volume was obtained using 1 dose of EtOH in the 4 hours and permanent
Cerebral Metabolic Disorder and Its Attenuation
Rats in the 4-hour MCAO group showed a significant \((P<0.01)\) increase in ADP/ATP ratio at 3 and 24 hours of reperfusion (Figure 3A), suggesting a reduced energy production. The 2-dose EtOH therapy was able to further \((P<0.01)\) decrease this ratio. This ratio was also significantly \((P<0.01)\) elevated in permanent stroke (Figure 3B). Conversely, the monotherapy of NBO or EtOH only mildly \((P<0.05)\) reduced this ratio as compared with the no-treatment group. In contrast, EtOH and 95% NBO therapy additively \((P<0.01)\) decreased this ratio.

Oxidative Stress
As compared with sham-operated group, ischemia for 4 hours significantly \((P<0.01)\) increased ROS production at 3 and 24 hours of reperfusion (Figure 4A). In tandem EtOH doses significantly \((P<0.01)\) reduced ROS levels. Likewise, a significant \((P<0.01)\) increase in ROS levels was observed in the permanent stroke (Figure 4B). Neither NBO nor EtOH alone was able to significantly decrease ROS. However, combined treatment induced a large \((P<0.01)\) reduction in ROS levels, suggesting an attenuated oxidative damage.

PDHC and PDK Protein Expression
Compared to the sham-operated group, 4-hour ischemia followed by 3-hour reperfusion caused a decrease \((P<0.05)\) in PDHC protein expression (Figure 5A). This decrease was even more significant \((P<0.01)\) at 24-hours reperfusion. EtOH was able to restore PDHC protein expression back to normal levels \((P<0.01)\) at both time points. Permanent stroke significantly \((P<0.01)\) reduced PDHC expression (Figure 5B). Although EtOH or NBO alone were only able to slightly reverse this reduction, EtOH nevertheless raised the level of PDHC expression better than NBO. In contrast, the EtOH+NBO combination substantially \((P<0.01)\) raised PDHC expression. In correlation with decreased PDHC, PDK protein expressions were increased \((P<0.01)\) in ischemic rats with 4-hour MCAO followed by 3- and 24-hour reperfusion, respectively (Figure 5C). In tandem EtOH doses significantly \((P<0.01)\) decreased PDK expression. Likewise, permanent MCAO significantly \((P<0.01)\) increased PDK (Figure 5D). In addition, the monotherapy with NBO or EtOH only mildly reduced PDK levels. Similarly to its effects on PDHC, EtOH alone induced greater reduction in PDK as compared with NBO alone. As above, the combined treatment was successful in decreasing these levels \((P<0.01)\).

PDP Protein Expression
Compared to sham-operated control, PDP1 protein expression after 4-hour MCAO was hardly changed at 3 and 24 hours of reperfusion (Figure 6A), whereas EtOH significantly \((P<0.01)\) increased PDP1 levels. After permanent stroke, PDP1 expression was slightly increased (Figure 6B). Although EtOH but NBO alone slightly altered PDP1 expression, EtOH+NBO combination significantly \((P<0.01)\) increase PDP1. In addition, we assessed another PDP protein level, that of PDP2 (Figure 6C and D). The results further confirm the beneficial effects of EtOH alone or EtOH in

MCAO groups. Furthermore, when 2 doses of EtOH were used, a significant \((P<0.01)\) reduction in infarct volume was observed in the 4-hour MCAO ischemic group (Figure 1B). In the permanent stroke groups, infarct volume \((#P<0.05;\) Figure 1C) was only mildly reduced by either monotherapy with EtOH (although 2 doses) or 95% NBO (Figure 1A and C). The most significant reduction \((P<0.01)\) in brain infarction was obtained when EtOH and 95% NBO were combined (Figure 1C). Examples of 2, 3, 5-triphenyltetrazolium chloride histology show the reduction in penumbral region of ischemic territory supplied by MCA as hypothesized (Figure 1A–C). In addition, compared with control, neurological deficits in both the 2- and 3-hour MCAO groups were decreased significantly \((P<0.01)\) after single EtOH treatment (Figure 2A). However, neither a single EtOH dose in 4-hour MCAO (Figure 2B) nor in tandem EtOH doses in permanent stroke (Figure 2C) were sufficient to reduce neurological deficits (\(P=0.068\)). Similarly after permanent MCAO, EtOH+NBO therapy again most effectively \((P<0.01)\) diminished the neurological deficits.
combination with NBO in both the transient (4-hour MCAO) and permanent stroke.

**Discussion**

**EtOH, NBO, and Neuroprotection**

This study revealed a dose-dependent neuroprotection of EtOH after transient ischemia. In permanent stroke, this neuroprotection was augmented when EtOH was therapeutically combined with NBO. Because reperfusion therapy within a clinically realistic time window is not available to most patients with stroke, a goal of the study was to assess EtOH and NBO neuroprotection under severe ischemic conditions with longer time frames, in contrast to those used in a previous study with moderate stroke (2-hour occlusion or less) and shorter therapeutic window.9,17 Our analysis after treatment with EtOH alone in transient ischemia or NBO+EtOH with permanent ischemia improved ATP production and reduced ROS generation. Although the measures here cannot definitely explain the better outcomes, our findings support a role of EtOH in stabilizing dysfunctional metabolic pathways mediated by PDHC, PDK, and PDP in ischemia. In addition, NBO may enhance EtOH effects by ameliorating ischemia-induced anaerobic metabolism and attenuating the generation of ROS. In this study, the infarct volume among the brains was variable; the dissected tissues may have included some undamaged tissue, which perhaps would have been rescued by the treatments. In fact, most of our treatments' effects were seen in the penumbra region.

Oxygen has been used in the treatment of stroke as a logical approach to counteract the hypoxic state induced by ischemia.3 Clinically, NBO has been shown to protect the ischemic brain from damage.20–24 However, in both transient and permanent focal ischemia, NBO has a relatively short time window and low therapeutic effect.3,10 The recent clinical trial for NBO in acute ischemic stroke was terminated because of unclear efficacy. There was a high mortality rate in the controlled arm of the study that was attributed to external causes (http://clinicaltrials.gov/show/NCT00414726). Thus, it is possible that NBO alone may not fully resolve ischemic injury because of its multifactorial, deleterious mechanisms. Although EtOH therapeutic potential lies on its ability to ameliorate the metabolic and oxidative distress during ischemia, especially in the case of short transient ischemia as confirmed by us.8,9 Our present results show that EtOH alone was no longer effective in ischemia without reperfusion. The addition of oxygen as an oxidative compliment is thus deemed essential to confer neuroprotection. Finally, this study also reveals that, although when used alone EtOH effect in permanent stroke was minor, it produced better outcome than NBO on ROS, as well as on PDHC, PDK, and PDP expressions. The mechanism by which EtOH achieves its effect may be because of its ability to induce a hibernation state and minimize oxidative damage by partially inhibiting glycolysis and other metabolic pathways.7,25 These effects in turn may slow down energy depletion and extend the survival time of cells in the penumbra region.26 Thus when metabolic dysfunction was inhibited by EtOH, oxygen may be better able to ameliorate the hypoxic state of cells.

**PDHC Mechanism in Ischemia**

PDHC is regarded as a central regulatory site of cellular oxidative metabolism in the mitochondrial matrix. PDHC sits...
at the cross-roads of the aerobic citric acid cycle, oxidative phosphorylation, anaerobic glycolysis, and gluconeogenesis. PDHC is a multienzyme complex composed of 6 subunits and 2 regulatory kinase molecules, PDK and PDP. Any dysfunction of PDHC and its regulators would result in metabolic crisis, such as a decline in ATP and phosphocreatine, inability to generate nicotinamide adenine dinucleotide and accumulation of lactic acid. Specifically, targeted regulation of PDHC occurs primarily via site-specific phosphorylation. After ischemic stroke, a severe oxygen deprivation leads to a large impairment of oxidative phosphorylation, thus limiting the production of ATP. This, in turn, raises the production of ROS after reperfusion which exacerbates cell and tissue injuries. Our present results support previous study, where large increase in ADP/ATP ratio led to significant rise in ROS production, which then would further augments ROS production in mitochondria. Using ATP production as an index here, decreased ROS generation by EtOH in transient or EtOH+NBO in permanent stroke suggests an overall better cellular metabolic status. We, therefore, would advance that EtOH and NBO may be clinically useful to restabilize cellular metabolism by modulating 2 pathways of ROS damage—the direct pathway for ROS generation and an indirect one for ROS generation via PDHC.

**PDHC and Its Regulators**

Because PDK and PDP are major regulators of PDHC, another major goal of the study was to assess how these regulators might be affected in severe ischemia, and how EtOH and NBO therapies could affect the regulators. Our results on increase in PDK protein expression after ischemia confirm previous studies showing a rise in PDK expression from ischemia as a signal of low energy state pushing the cell to use fatty acids or ketone bodies as a major energy source. Our therapy decreased the levels of PDK protein, which led to disinhibition of PDHC.
by increasing its expression, thus helping to stabilize the cell after ischemic insult. In addition, we observed a small increase in the expression of PDP1 and PDP2 after ischemia, even before treatment. These findings stand in contrast to the PDP isoenzyme declines previously observed after traumatic brain injury. Such increases could be a mild compensatory mechanism whereby the cell attempts to self-modulate and stabilize the concentration of PDHC, although this compensatory mechanism seems to be insufficient. Our therapy (EtOH or EtOH+NBO), however, was able to further increase PDP levels beyond its compensatory physiological levels, leading to neuroprotection. Taken together, these results strongly support the concept that anaerobic metabolism after stroke may have been stabilized through the effects of PDHC expression by EtOH or EtOH+NBO which led to decreased PDK, whereas simultaneously increasing PDP levels.

Clinical Potential of EtOH and NBO Therapy
Collateral perfusion, widely recognized to remain functional after stroke, may exert a dramatic effect on the time course of ischemic injury, stroke severity, imaging findings, as well as therapeutic opportunities and subsequent neurological outcomes after stroke. Because EtOH and NBO are widely available, readily cross the blood brain barrier and easily diffuse through the collateral circulation into ischemic regions, even before reperfusion is established, their clinical potential as therapeutics is apparent. Future implementation of EtOH and NBO in a clinical setting may move us closer toward the development of an efficacious neuroprotective therapy.

Although the primary focus here is centered on treatment effects on ischemic brain tissues, additional mechanistic studies are needed to assess the correlation between functional outcomes and the regulatory roles of PDP and PDK. At a more fundamental level, future studies will also aim to determine the cause/effects relations between EtOH/NBO outcomes and PDHC and its regulators in ischemic as well as nonischemic brain tissues.

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**References**


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**SUPPLEMENTAL MATERIAL**

**Materials & Methods**

**MCA Occlusion (with additional information).** Rats were subjected to MCAO for either 2, 3, 4, or 28 h using the intraluminal filament mode\(^1\). Briefly a 4.0 nylon suture with a blunted tip coated with poly-L-lysine was inserted into the right external carotid artery and lodged in the narrow proximal anterior cerebral artery to block the MCA at its origin. Reperfusion was achieved by the withdrawal of the filament at each corresponding time point as mentioned above. Blood p\(\text{CO}_2\) and p\(\text{O}_2\), mean arterial pressure (MAP), as well as rectal and brain temperature were monitored throughout the procedure. In addition, heating lamps and pads were utilized to maintain rectal temperature at 36.5-37.5 °C.

**ADP/ATP Ratio (with additional information).** Brain metabolism was measured with the BioVision ApoSENSOR Assay Kit (CA, USA)\(^2\). Briefly right cerebral hemispheres of the rats were extracted and homogenized in cold PBS buffer. Next, the sample (10 µL) was transferred into a luminometer plate and 100 µL of the Nucleotide Releasing Buffer was added. The mixture was then incubated for 10 min at room temperature with gentle shaking and brain ATP levels in the brain were measured by adding 1 µL of the ATP Monitoring Enzyme into the brain cell lysate. A luminometer (DTX 880 Multimode Detector, Beckman Coulter) was used to read the samples after 1 min (Data A). After 10 mins, ADP levels were measured again (Data B). One µL of ADP Converting Enzyme was then added and the samples were read once more after 1 min (Data C). ADP/ATP ratio was calculated as: (Data C – Data B)/Data A.

**ROS Production (with additional information).** The method for detection of ROS was described previously by us\(^3\). This method tests \(\text{H}_2\text{O}_2\) levels with hydrogen peroxidase linked to a fluorescent compound. Brain samples taken from the animals were diluted to 10 mg/ml based on protein concentration (BCA method) and incubated for 30 min after 100 µg/ml of digitonin addition. \(\text{H}_2\text{O}_2\) levels in brain homogenates were determined using 50 µM Amplex red, 0.1 U/ml horse radish peroxidase (HRP), and respiratory substrates (4 mM pyruvate, 2 mM malate, 2 mM glutamate, and 0.8 µM complex V inhibitor oligomycin) at 37°C on a DTX-880 multimode detector.

**Protein Expression (with additional information).** Western Blot analysis was used to detect protein levels of PDHC, PDP, and PDK. Briefly, extracted protein from the brain issue of fronto-parietal cortex and striatum were loaded onto a single 10% sodium dodecyl sulfate-polyacrylamide gel for electrophoresis. Forty µg of protein was loaded per well. Samples were transferred to a polyvinylidene fluoride (PVDF) membrane (Bio-Rad, Hercules, CA). Primary antibody incubation (PDHC, 1:250; PDP1, 1:1000; PDP2, 1:1000; PDK, 1:1000, Santa Cruz Biotechnology, Dallas, Texas) was then carried out overnight at 4°C. Secondary antibody incubation (anti-goat, anti-rabbit, 1:1250; 1:5000; 1:5000; 1:5000, Santa Cruz Biotechnology, Dallas, Texas) was done for one hour at room temperature. An ECL system was used to detect immunoreactive bands by luminescence and to quantify protein expression profiles, with the relative density of Western blot images being obtained by using an image analysis program (ImageJ 1.48, National Institutes of Health, USA). As a reference, the mean amount of protein expression from the sham-operation group was assigned a value of 1. The expressions of target proteins were represented as fold-differences compared to the control.
Results

Physiological parameters (Tables I and II). There were no significant difference in blood pH and pCO$_2$ in each group. However, a significantly ($P<0.05$) increased arterial blood pO$_2$ was found in the NBO-treated groups. Body and brain temperature remained at about 37 °C.

Table 1. Physiologic Parameters during Transient MCA Occlusion.

<table>
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<tr>
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<th>Stroke (2h)</th>
<th>Stroke (2h) and EtOH (1.5g/kg)</th>
<th>Stroke (3h)</th>
<th>Stroke (3h) and EtOH (1.5g/kg)</th>
<th>Stroke (4h)</th>
<th>Stroke (4h) and EtOH (1.5+1.0g/kg)</th>
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<td>2h after reperfusion</td>
<td>41.1±5.7</td>
<td>49.3±2.3</td>
<td>43.0±1.0</td>
<td>46.6±4.5</td>
<td>45.2±2.3</td>
<td>42.1±3.5</td>
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</tbody>
</table>
### Table II. Physiologic Parameters During Permanent MCA Occlusion.

<table>
<thead>
<tr>
<th></th>
<th>Stroke</th>
<th>Stroke and EtOH (1.5g/kg)</th>
<th>Stroke and EtOH (1.5+1.0g/kg)</th>
<th>Stroke and NBO</th>
<th>Stroke and EtOH (1.5+1.0g/kg)/NBO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MAP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre MCAO</td>
<td>84.2±2.1</td>
<td>87.6±3.1</td>
<td>87.4±2.2</td>
<td>90.1±2.6</td>
<td>84.8±2.3</td>
</tr>
<tr>
<td>2h after MCAO</td>
<td>89.1±3.3</td>
<td>82.6±2.7</td>
<td>85.5±2.7</td>
<td>83.3±4.2</td>
<td>87.0±2.6</td>
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<tr>
<td>8h after MCAO</td>
<td>86.2±2.9</td>
<td>83.6±2.2</td>
<td>89.1±3.7</td>
<td>84.3±2.8</td>
<td>82.2±2.9</td>
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<tr>
<td><strong>pH</strong></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Pre MCAO</td>
<td>7.39±0.02</td>
<td>7.35±0.05</td>
<td>7.36±0.02</td>
<td>7.40±0.03</td>
<td>7.38±0.02</td>
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<tr>
<td>2h after MCAO</td>
<td>7.41±0.02</td>
<td>7.36±0.02</td>
<td>7.37±0.01</td>
<td>7.37±0.03</td>
<td>7.39±0.02</td>
</tr>
<tr>
<td>8h after MCAO</td>
<td>7.35±0.02</td>
<td>7.37±0.01</td>
<td>7.40±0.03</td>
<td>7.35±0.02</td>
<td>7.40±0.01</td>
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<tr>
<td><strong>PO₂</strong></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Pre MCAO</td>
<td>134.9±5.7</td>
<td>138.9±6.1</td>
<td>133.9±4.3</td>
<td>132.9±7.5</td>
<td>132.5±5.2</td>
</tr>
<tr>
<td>2h after MCAO</td>
<td>131.5±6.7</td>
<td>134.9±4.4</td>
<td>138.3±5.1</td>
<td>134.9±6.1</td>
<td>141.6±7.4</td>
</tr>
<tr>
<td>8h after MCAO</td>
<td>139.1±7.7</td>
<td>135.8±6.2</td>
<td>129.1±7.8</td>
<td>419.6±19.1*</td>
<td>434.1±19.9*</td>
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<tr>
<td><strong>PCO₂</strong></td>
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</tr>
<tr>
<td>Pre MCAO</td>
<td>45.5±1.6</td>
<td>47.1±1.9</td>
<td>45.3±2.1</td>
<td>48.0±2.0</td>
<td>44.4±1.8</td>
</tr>
<tr>
<td>2h after MCAO</td>
<td>43.9±2.7</td>
<td>47.8±1.5</td>
<td>44.8±2.2</td>
<td>43.3±2.5</td>
<td>46.0±2.2</td>
</tr>
<tr>
<td>8h after MCAO</td>
<td>48.1±4.3</td>
<td>43.9±2.2</td>
<td>47.4±1.9</td>
<td>43.1±2.3</td>
<td>46.4±1.9</td>
</tr>
</tbody>
</table>

Physiologic parameters of Mean Arterial Pressure (MAP), blood PCO₂, PO₂, and pH were recorded before, during, and after permanent MCA occlusion. * indicates P<0.01 as compared to other groups.

**Reference**