Effect of Normobaric Oxygen Therapy in a Rat Model of Intracerebral Hemorrhage

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Background and Purpose—Normobaric oxygen (NBO) therapy may be neuroprotective in acute ischemic stroke. However, how NBO may affect intracerebral hemorrhage is unclear. We tested NBO in a rat model of striatal intracerebral hemorrhage.

Methods—Intracerebral hemorrhage was induced by stereotactic injection of collagenase Type VII (0.5 U) into the right striatum of male Sprague-Dawley rats. One hour later, rats were randomized into controls (n=13) versus NBO treatment (n=13). NBO was applied for 2 hours. Hemorrhagic blood volume, brain water content, and neurological outcomes (forelimb placement test, forelimb asymmetry, neuroscore) were quantified at 72 hours. Experiments were repeated in a second independent laboratory to assess reproducibility in neurological outcomes (n=10 per group).

Results—NBO did not worsen hemorrhage severity or brain edema. There were no significant differences in hemorrhagic blood volumes (control, 6.4±0.9 μL versus NBO, 7.0±2.1 μL; P=0.18) or brain water content (control, 81.9%±1.1% versus NBO, 81.6%±0.5%; P=0.58). NBO did not affect any of the neurological outcome tests in the primary or secondary studies.

Conclusions—NBO therapy may not worsen outcomes in intracerebral hemorrhage. (Stroke. 2011;42:1469-1472.)

Key Words: acute stroke ■ intracranial hemorrhage ■ ischemia ■ neuroprotection

Early treatment is important for patients with stroke. In this regard, normobaric oxygen (NBO) has been proposed as a potential treatment that can be easily administered, even in the ambulance.1 However, 1 potential worry is that NBO may have adverse effects in intracerebral hemorrhage (ICH). We tested the effects of NBO in a rat model of collagenase-induced striatal hemorrhage.

Materials and Methods

Animal Model
All experiments were performed following an institutionally approved protocol in accordance with National Institutes of Health guidelines. ICH was induced by collagenase injections.2 Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were anesthetized with isoflurane (1% to 1.2%) in 30%/70% oxygen/nitrous oxide. A catheter in the right femoral artery was used for measuring blood pressure, pH, PaO₂, and PaCO₂. Using a stereotactic frame, 1 μL of saline containing 0.5 U collagenase VII-S (Sigma) was injected through a 22-gauge needle over 5 minutes into the right striatum (from bregma: 1.2 mm anterior, 3.5 mm lateral, 5.3 mm depth). One hour later, rats were randomized into NBO (100% oxygen through a face mask for 2 hours) versus control groups. Two separate studies were performed. In the primary study (Massachusetts General Hospital, Boston, MA), 13 rats per group were used. In the secondary study (Xuanwu Hospital, Beijing, China), 10 rats per group were used.

Hemorrhagic Volume
Sixteen rats (n=8 per group) were euthanized at 72 hours in the primary Massachusetts General Hospital study. Brains were perfused with phosphate-buffered saline. Hemorrhagic volumes were measured using a spectrophotometric assay for hemoglobin.3 Calculations were performed with blinding.

Brain Water Content
Ten rats (n=5 per group) were euthanized at 72 hours without perfusion in the primary Massachusetts General Hospital study. Brain water content was assessed using standard methods.4 A single 4-mm section was cut centered around the hematoma, and edema was calculated as: [(wet weight–dry weight)/wet weight]×100%. Calculations were performed with blinding.

Neurological Tests
For both primary (Massachusetts General Hospital) and secondary (Xuanwu) studies, all rats were assessed blindly at 72 hours using 3 tests: forelimb placement, forelimb asymmetry, and a 5-point neuroscore scale.5,6 Vibrissae-elicited forelimb placement was averaged over 10 times for each forelimb. Forelimb asymmetry was analyzed...
by videotaping rats in a transparent cylinder for 10 minutes. Neuroscores were graded as 0=no apparent deficit; 1=slight deficit; 2=circling; 3=heavy circling or no movement at all; or 4=death.

Statistics
Hemorrhagic volume and water content were compared with unpaired t tests. Behavioral outcomes were compared with Mann-Whitney U tests.

Results
Except for PaO\textsubscript{2}, all physiological parameters remained within the normal range in both groups (Table). NBO elevated PaO\textsubscript{2} levels to 400 mm Hg (Table). This collagenase model yielded well-defined striatal hematomas (Figure 1A). NBO did not worsen hemorrhage volumes (control, 6.4±0.9 mL versus NBO, 7.0±2.1 mL; \( P=0.18 \); Figure 1B).

Brain water content in the ipsilateral hemisphere was significantly greater than the contralateral hemisphere in both groups (NBO group: ipsilateral, 81.6%±0.5% versus contralateral, 79.2%±0.3%; \( P<0.01 \); control group: ipsilateral, 81.9%±1.1% versus contralateral, 79.4%±0.4%; \( P<0.01 \); Figure 1C). However, NBO did not worsen brain edema (NBO ipsilateral, 81.6%±0.5% versus control ipsilateral, 81.9%±1.1%; \( P=0.58 \); Figure 1C).

NBO did not worsen neurological outcomes in the primary (Massachusetts General Hospital) study. Forelimb placement was significantly affected by ICH (NBO group: contralateral, 21.5%±11.4% versus ipsilateral, 88.5%±13.4%; \( P<0.01 \); control group: contralateral, 14.6%±8.8% versus ipsilateral, 86.1%±9.6%; \( P<0.01 \); Figure 2A). However, there was no difference between NBO and control rats (Figure 2A). NBO had no effect on forelimb asymmetry (NBO group: 26.0%±38.6% versus control group: 31.1%±32.6%; Figure 2B). NBO did not affect mortality or neuroscore (Figure 2C).

Similar findings were obtained in the secondary (Xuanwu Hospital) study. NBO did not worsen outcomes in forelimb asymmetry (Figure 3A), forelimb placement (Figure 3B), or overall neuroscore (Figure 3C).

Discussion
NBO has been proposed as a therapeutic approach to lengthen reperfusion windows in acute ischemic stroke.\textsuperscript{1} In theory, NBO could even be rapidly initiated without a diagnostic

<table>
<thead>
<tr>
<th>Table. Physiological Parameters</th>
<th>NBO</th>
<th>Control</th>
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<tbody>
<tr>
<td>Body wt, g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>299.2±30.3</td>
<td>299.2±17.9</td>
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<tr>
<td>Day 3</td>
<td>290.4±28.5</td>
<td>285.6±24.0</td>
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<tr>
<td>Weight loss</td>
<td>8.8±7.1</td>
<td>13.5±13.3</td>
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<tr>
<td>Rectal temperature, °C</td>
<td></td>
<td></td>
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<tr>
<td>Pre-ICH</td>
<td>37.1±0.1</td>
<td>37.1±0.2</td>
</tr>
<tr>
<td>1 hour</td>
<td>37.1±0.1</td>
<td>37.0±0.1</td>
</tr>
<tr>
<td>1.5 hours</td>
<td>37.1±0.2</td>
<td>37.0±0.1</td>
</tr>
<tr>
<td>PaO\textsubscript{2}, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-ICH</td>
<td>138.6±7.9</td>
<td>136.2±6.0</td>
</tr>
<tr>
<td>1 hour</td>
<td>132.2±9.0</td>
<td>135.2±5.7</td>
</tr>
<tr>
<td>1.5 hours</td>
<td>459.4±32.4</td>
<td>133.5±7.6</td>
</tr>
<tr>
<td>PaCO\textsubscript{2}, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-ICH</td>
<td>48.3±3.6</td>
<td>46.8±5.2</td>
</tr>
<tr>
<td>1 hour</td>
<td>47.7±5.2</td>
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<td>1.5 hours</td>
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<td>pH</td>
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<tr>
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<td>7.42±0.03</td>
<td>7.44±0.04</td>
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<tr>
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<tr>
<td>1.5 hours</td>
<td>7.39±0.05</td>
<td>7.39±0.04</td>
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</table>

Values are mean±SD. NBO indicates normobaric oxygen; ICH, intracerebral hemorrhage.
distinction between ischemic versus hemorrhagic strokes. Here, we tested the effects of NBO in a rat ICH model. There were no protective effects. However, NBO did not worsen outcomes after ICH.

NBO is beneficial in experimental models of cerebral ischemia. However, to our knowledge, NBO has never been tested in ICH. Because there are many overlapping mechanisms in ischemia and hemorrhage, it was theoretically possible that NBO could also be protective after hemorrhage. However, we were unable to detect any beneficial effects in our study. Of course, this was primarily designed as a safety study so we restricted treatment to only 2 hours post-ICH. Whether longer exposures to NBO will yield benefit for ICH remains to be determined.

A major worry with NBO is the fear of generating free radicals and worsening neuronal injury. For acute ischemic stroke, NBO did not appear to augment radical generation or worsen markers of oxidative damage after cerebral ischemia. Nevertheless, oxidative stress may contribute to secondary injury surrounding a hematoma. The free radical scavenger edaravone reduced acute ICH-induced brain edema and neurological deficits. However, here, we did not observe any worsening of hemorrhage or brain edema after ICH. Hence, our findings suggest that at least in the context of short-term treatments, NBO may not exacerbate injury after ICH. A recent study showed that NBO may even reduce rates

Figure 2. Neurological outcomes after ICH in the primary (Massachusetts General Hospital [MGH]) study. A, Contralateral forelimb placement (mean±SD) showed significant deficits compared with ipsilateral forelimb in both control and NBO groups. *P<0.01. However, NBO did not worsen this deficit. B, NBO had no significant effect on the forelimb use asymmetry score (mean±SD). C, NBO did not affect mortality or neurological severity. ICH indicates intracerebral hemorrhage; NBO, normobaric oxygen.

Figure 3. Neurological outcomes after ICH in the secondary (Xuanwu) study. A, Contralateral forelimb placement (mean±SD) showed significant deficits compared with ipsilateral forelimb in both control and NBO groups. *P<0.01. However, NBO did not worsen this deficit. B, NBO had no significant effect on the forelimb use asymmetry score (mean±SD). C, NBO did not affect mortality or neurological severity. ICH indicates intracerebral hemorrhage; NBO, normobaric oxygen.
of hemorrhagic conversion after thrombolysis in embolic focal cerebral ischemia in rats. However, future studies should carefully assess whether longer exposures to NBO may influence the pathophysiology of excitotoxicity, oxidative stress, inflammation, and cell death after hemorrhage. A final caveat involves the nature of our animal model. There are no perfect models for ICH. Although this collagenase-induced ICH approach is widely used, and produces vascular trauma along with reproducible hematomas, it does not replicate all aspects of ICH in human patients. In this model, hematomas rapidly grow over 3 to 4 hours postcollagenase and tend to maximize by 5 to 6 hours. Whether NBO affects hemorrhages that develop over longer periods of time remains unknown.

In conclusion, NBO did not worsen outcomes in a rat model of ICH. These initial safety data suggest that NBO could potentially be started even before a definitive diagnosis is made to distinguish ischemic versus hemorrhagic stroke. However, of course, these experimental results require clinical confirmation.

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Disclosures
None.

References
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