Retrograde Transvenous Neuroperfusion
A Back Door Treatment for Stroke

John G. Frazee, MD; Xia Luo, MD; Guoming Luan, MD, PhD; David S. Hinton, MD; David A. Hovda, PhD; Mark S. Shiroishi, BS; Larry T. Barcliff, RRT

Background and Purpose—Stroke is the third leading cause of death and the leading cause of adult disability in the United States. The clot-lysis drug tissue plasminogen activator is the only treatment that has been effective for acute stroke patients, yet there are significant limitations to its use and effectiveness. In this study retrograde transvenous neuroperfusion (RTN) was evaluated for its efficacy in reversing acute ischemia, preventing paralysis, and limiting pathological evidence of infarction in baboons.

Methods—Ten adult male baboons underwent 3.5 hours of reversible middle cerebral artery occlusion (MCAO) under isoflurane (0.25% to 1.5%) anesthesia. Five randomly chosen animals received RTN treatment 1 hour after start of MCAO. Somatosensory evoked potentials were recorded during MCAO. Animals were assigned daily neurological scores. Animals were killed 6 days after MCAO, and brains were quantitatively analyzed for infarct volume.

Results—Within 1 hour after RTN was started, treated animals showed significantly improved somatosensory evoked potentials (103.3% versus 75% of baseline; \( P < 0.01 \)). Likewise, the combined neurological score for the RTN-treated group was 99.2, while the combined mean score for the untreated group was 66.4 (\( P < 0.015 \)). The mean infarction volume was 8.8 ± 3.1% (of contralateral hemisphere) for the control group and 0.3 ± 0.2% for the RTN-treated group (\( P < 0.01 \)). No increased mortality was seen in the RTN-treated group.

Conclusions—We conclude that RTN treatment during MCAO effectively reverses the pathophysiological sequelae of ischemia, even when the treatment is initiated 1 hour after the onset of ischemia. Although the infarct volume in the control group was variable when quantitatively assessed 6 days after 3.5 hours of MCAO, virtually no evidence of infarcts was seen in the RTN-treated group. (Stroke. 1998;29:1912-1916.)

Key Words: cerebral ischemia ■ middle cerebral artery occlusion ■ perfusion ■ stroke, experimental ■ baboons

For decades, investigators have unsuccessfully sought an effective early treatment for stroke.\(^1\) Stroke is the third leading cause of death in the United States, affecting >500,000 people each year. It is the leading cause of adult disability, with costs reaching >$30 billion annually.\(^2\)\(^-\)\(^4\)

Eighty percent of strokes are ischemic, resulting from an arterial obstruction. This obstruction is most commonly caused by a blood clot, which has led to the use of clot-lysis drugs such as tissue plasminogen activator (tPA).\(^5\) However, tPA use has important limitations, including a potentially significant time to achieve clot lysis, 3-hour treatment window from the onset of symptoms, and nearly 6% occurrence of cerebral hemorrhage.\(^5\) Another approach to stroke treatment has been the reliance on collateral circulation for the delivery of drugs that may reduce or prevent cellular injury.\(^6\)\(^-\)\(^7\) The use of the collateral circulation depends on an indirect pathway for delivery of these drugs, a pathway that is neither efficient nor completely effective.\(^8\)

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needed to maintain the activated clotting time at

cally supplemented with one third of the original dose of heparin as

and left transverse sinuses adjacent to the torcular (Figure 1B). All

for arterial blood. Two catheters were surgically placed in the right

MCAO with a transorbital approach and an aneurysm clip (Figure

pressures were maintained between 8 and 15 mm Hg. The mean

measured at the catheter tips (Neuroperfusion, Inc). Intrasinus

Maximum flow rates were determined by intrasinus pressures

in the transverse sinuses and directed retrograde by the partial

obstruction of the sinuses by the catheter and by the flow rate.

plished by pump-controlled flow rates and by partial obstruction

of the venous sinuses using the catheter itself or variably inflatable

balloons at the catheter tips. Pressures only moderately above normal venous pressures and well within acceptable limits are all that are necessary to drive the blood toward the ischemic tissue. The blood presumably traverses the capillary bed to exit through the redundant venous system.

In this study we hypothesized that RTN treatment, initiated 1 hour after middle cerebral artery occlusion (MCAO), would reverse the effects of prolonged ischemia as assessed by somatosensory evoked potentials (SSEPs), neurological evaluations, and quantitative neuropathological analysis in baboons.

Materials and Methods

Ten adult male baboons (Papio ursinus) weighing 20 to 30 kg were studied. Their care and treatment were in accordance with institutional guidelines. All animals were premedicated with ketamine (7.5 mg/kg IM) and atropine (0.04 mg/kg IM), then intubated, paralyzed with pancuronium bromide (Pavulon, 0.1 mg/kg IV), and placed on a respirator delivering a tidal volume of 10 mL/kg. Isoflurane (0.25% to 1.5%) and nitrous oxide (60%) were used for continuous anesthesia. Animals were monitored for ECG, blood pressure, end-tidal CO₂, oxygen saturation, electroencephalography, and SSEPs.

Focal reversible ischemia was induced for 3.5 hours by right MCAO with a transorbital approach and an aneurysm clip (Figure 1A). All animals had catheters placed in 1 femoral arterial source for arterial blood. Two catheters were surgically placed in the right and left transverse sinuses adjacent to the torcular (Figure 1B). All animals were fully anticoagulated (heparin, 100 U/kg) and periodically supplemented with one third of the original dose of heparin as needed to maintain the activated clotting time at >300 seconds.

Five randomly chosen animals (RTN-treated group) received RTN treatment beginning 1 hour after the start of ischemia and continued for the final 2.5 hours of arterial occlusion. The RTN treatment was initiated 1 hour after MCAO since that was the length of time typically required to expose the sinus and catheterize the femoral artery. For RTN treatment, arterial blood was harvested from a femoral artery by an external pump and passed through the 2 catheters. Blood was directed retrograde through the sagittal as well as other sinuses (not shown); ICA indicates internal carotid artery; A-1 segments, the right and left anterior cerebral arteries before becoming a single A-2 segment.

resting intrasinus pressure during ischemia was 2.8 mm Hg for the RTN-treated group (5.0 mm Hg for the control group). The mean intrasinus pressure during RTN treatment was 9.8 mm Hg. RTN was continued for the last 2.5 hours of the 3.5 hours of ischemia. Five additional animals (control group) were subjected to the same treatments as the RTN-treated group except that retrograde arterial blood flow was never started. After 3.5 hours, aneurysm clips were removed from all animals in both groups to permit reperfusion. All surgical sites were closed, and animals were awakened. Each experiment typically lasted <5 hours. Animals were observed daily, and neurological examinations were scored by 2 blinded observers using a system similar to human stroke scores (Table 1). The scores between the 2 observers, which did not deviate by >5 to 15 points (out of 100), were averaged for each day.

Grass subdermal needle electrodes were positioned over the median nerves. For each side, the anode was 1 cm from the wrist crease and the cathode was 1.5 cm proximal to the anode. The SSEP was elicited as a 0.2–0.1 millisecond constant-current square-wave peak given to the left and right median nerves separately. The stimulus presentation rate was 4 Hz. Cortical SSEPs were recorded from Grass subdermal needles placed in the scalp overlying the contralateral (to the evoking stimulus) somatosensory cortex. Subcortical SSEPs were recorded with the use of a subdermal electrode placed over the second cervical vertebra. Bilateral subdermal electrodes were also placed at Erb’s point. The cortical and subcortical electrodes were both referenced to a subdermal electrode placed over the midline 5 cm behind the orbital rim. Ground electrodes were placed on both ears and connected together. SSEP recording was continuously performed by a recorder (Axon System, Inc) over an analysis time of 40 milliseconds. In all experiments, a 40% decrease from baseline SSEPs signified the presence of ischemia.

Animals were killed on the sixth day after the experiment, and their brains were removed and immersion fixed in 10% buffered formalin for 4 weeks. For each case, coronal sections (75 μm) were cut through the middle cerebral artery (MCA) territory at 1-mm intervals and stained for hematoxylin and eosin. Each coronal section was digitized on a high-resolution scanner (Epson 636) and stored on a CD-ROM. These digitized images were assessed by the neuropathologist (D.S.H.), who was blinded as to the treatment of each animal. Infarcted areas were traced with the aid of a computer on the basis of optical density.
differences, and volumes were calculated by multiplying infarct areas of each brain slice by the thickness of each slice. Optical densities for infarcted areas showed a decrease in optical density of >40% compared with surrounding tissue. The resulting volumes for all slices from the right hemisphere were combined and expressed as a percentage of the contralateral hemisphere volume.

Data Analysis
All values are expressed as mean±SEM. Statistical analysis for SSEPs was conducted with one-way ANOVA by group and time, with percent change from baseline levels as within-subject variable. Neurological evaluations (stroke scores) between treated and untreated groups over 6 days were compared with the Kruskal-Wallis test. Comparisons of vital signs and infarct size for treated and non-treated control groups were made with the 2-tailed Student’s t test. Values of P<0.05 were considered significant.

Results
Vital signs remained stable throughout all experiments, except that systolic and mean arterial blood pressures began to rise in each animal after MCAO (Table 2). Nitroprusside (1 to 5 μg/kg per minute) was intravenously infused to maintain a mean systolic pressure of 170 mm Hg and a mean arterial pressure of 115 mm Hg. Ventilation was adjusted during the experiment to maintain normal blood chemistry values (Table 3). The start of RTN, by design, raised intrasinus pressures to higher than resting levels but no greater than 20 mm Hg. Intracranial pressures followed the intrasinus pressures in a delayed fashion, and values for both pressures were similar. After the termination of RTN, intrasinus and intracranial pressures returned to baseline levels.

During each experiment, SSEPs were observed to fall in amplitude after MCAO (Table 2). SSEPs were conducted with one-way ANOVA by group and time, with percent change from baseline levels as within-subject variable. Comparisons of vital signs and infarct size for treated and non-treated control groups were made with the 2-tailed Student’s t test. Values of P<0.05 were considered significant.

Daily stroke scores were averaged for the 6-day posttreatment period. Three untreated animals were slow to improve and had substantially lower scores during their recovery, while 2 showed no evidence of abnormal behavior. All RTN-treated animals were nearly normal on the first day after treatment. The combined results of the untreated group gave a mean stroke score for the 6 days of 66.4, while the combined mean score for the RTN-treated group was 99.2 (P<0.01) (Table 4).

Pathological outcome was consistent with that of the stroke score results. Major infarctions were present in 4 untreated animals with lowered neurological scores and were absent or insignificant in all RTN-treated animals, all of which had stroke scores of ≥98. The worst untreated animal had a 17% infarction (volume of infarction expressed as a percentage of the contralateral hemisphere) (Table 5). The 1 remaining untreated animal also had a detectable infarct localized to the right MCA territory, but it constituted only 2.5% of the contralateral hemisphere. For the RTN-treated group, only 2 animals showed any indications of infarction, but even in these cases, the infarct sizes (0.5% to 0.8%) were 3 to 4 times smaller than the most minor infarct seen without RTN treatment (Table 5; Figure 2). The mean infarction volume of the right hemisphere was 8.8±3.1% for the control group and 0.3±0.2% for the RTN-treated group (P<0.01).

Discussion
In this study the results from SSEPs, stroke scores, and infarct size consistently demonstrated a positive effect from RTN treatment. After 1 hour of ischemia, RTN treatment was begun and was able to produce recovery from ongoing ischemia as measured by SSEPs. Stroke scores used to monitor the recovery of these animals over the next 6 days demonstrated a rapid and nearly full (mean stroke score, 99.2) recovery for the treated animals. The untreated animals incompletely recovered (mean stroke score, 66.4). Finally, the volume of injured brain was greater for the untreated animals in which it was found and occurred in more animals than for the RTN-treated group.

### TABLE 2. Physiological Values From Control (n=5) and RTN-Treated (n=5) Animals During Treatment

<table>
<thead>
<tr>
<th></th>
<th>MABP, mm Hg</th>
<th>ICPO, mm Hg</th>
<th>ISP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>MCAO</td>
<td>MCAO+RTN</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>119.0±4.3</td>
<td>123.0±3.0</td>
<td>n/a</td>
</tr>
<tr>
<td><strong>Treated</strong></td>
<td>126.4±4.8</td>
<td>123.0±1.0</td>
<td>111.3±10.8</td>
</tr>
</tbody>
</table>

MABP indicates mean arterial blood pressure; ICPO, intracranial pressure; and ISP, intrasinus pressure. Values are mean±SEM.

### TABLE 3. Blood Chemistry Values From Control (n=5) and RTN-Treated (n=5) Animals During MCAO

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>PCO₂, mm Hg</th>
<th>PO₂, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>7.35±0.03</td>
<td>36.3±2.3</td>
<td>124.5±21.6</td>
</tr>
<tr>
<td><strong>Treated</strong></td>
<td>7.40±0.04</td>
<td>34.4±2.7</td>
<td>150.9±25.7</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

### TABLE 4. Results From Control (n=5) and RTN-Treated (n=5) Animals

<table>
<thead>
<tr>
<th>SSEPs, % Change From Baseline</th>
<th>1 h</th>
<th>2 h</th>
<th>3.5 h</th>
<th>Stroke Score*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>−27±9</td>
<td>−25±19</td>
<td>−21±12</td>
<td>66.4±14.0</td>
</tr>
<tr>
<td><strong>Treated</strong></td>
<td>−13±9</td>
<td>3.3±14†</td>
<td>72±61†</td>
<td>99.2±0.4†</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*At day 6 after experiment.
†P<0.01, treated vs control.
The infarct size for the control group after 3.5 hours of MCAO was somewhat variable. In fact, 1 of the 5 untreated control cases showed only minor signs of an infarction. For the remaining 4 animals, the range of infarct volumes was 5.3% to 17%, which is consistent with those reported by Young et al. In their investigation, 6-hour MCAO in anesthetized baboons, with the use of a similar transorbital approach and microvascular clips, resulted in 3% to 11% infarcted volume in only 6 of 10 cases. In addition, a recent study by Nehls et al was only able to demonstrate infarcted brain areas after a similar MCAO in 4 of 6 baboons. These authors and others concluded that the infarct size produced after MCAO is significantly greater and more consistent when unanesthetized animals are used. Their assertion is based on the following rationales: (1) anesthesia offers significant neuroprotection; (2) anesthesia induces marked recruitment from collateral vessels; (3) stress from immobilization in unanesthetized animals results in hypocapnia, which exacerbates the injury; and (4) long periods of stress induced in unanesthetized animals may increase blood-brain permeability. These studies support the conclusion that anesthetic levels predominantly influence the variability in infarct size in our untreated control group.

However, it is important to remember that the variability in infarct volume within the control group does not invalidate our findings, since no macroscopic or microscopic evidence of an infarction was seen in 3 of 5 cases after RTN treatment. The 2 remaining cases showed extremely small infarcts (Table 5; Figure 1B), which were 3 to 21 times smaller than any infarct seen in the control group.

Our data provide indirect evidence that the ischemic tissue maintained viability as a result of retrograde flow of femoral arterial blood to the ischemic capillary bed during the RTN treatment. In support of this hypothesis, Symon measured MCA and middle cerebral vein pressures in primates before and after MCAO. Before occlusion, pressure measured in the proximal MCA averaged 94 mm Hg, while middle cerebral vein pressure averaged 14 mm Hg. Assuming an \( \approx 80\% \) drop in pressure between arterial and capillary pressures, it would be predicted that pressures in the nonischemic capillary bed would range between 18 and 28 mm Hg. This is in keeping with actual capillary bed measurements of 18 to 22 mm Hg in more accessible tissue of the finger. We predicted, therefore, that when RTN reached a pressure near 20 mm Hg, blood would flow retrograde to normal brain perfusion. When Symon measured pressures during ischemia, he found that arterial pressures fell to a mean of 20.6 mm Hg and venous pressures fell to a mean of 9.4 mm Hg. The pressure in the ischemic capillary bed of a primate must therefore be intermediate between measured arterial and venous pressures, ie, 9.4 to 20.6 mm Hg. Thus, it would only be necessary to generate pressures slightly higher than that of the ischemic capillary bed to produce retrograde flow that would pass preferentially to the ischemic bed. The pressures measured in the transverse sinuses during experiments reported here averaged 10 mm Hg, which agrees well with our theoretical prediction.

The present study was designed to maximize the benefit of RTN by introducing a short time between the onset of arterial occlusion and the start of RTN treatment. Previous animal studies by others have suggested that the therapeutic window for the treatment of acute stroke may be much longer than 1

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**TABLE 5. Quantitative Measurements of Infarct Size From Control (n=5) and RTN-Treated (n=5) Animals**

<table>
<thead>
<tr>
<th>Infarct Volume, ( \text{mm}^3 )</th>
<th>% Contralateral Hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control cases</strong></td>
<td></td>
</tr>
<tr>
<td>B260</td>
<td>133.7</td>
</tr>
<tr>
<td>B304</td>
<td>356.1</td>
</tr>
<tr>
<td>WP244</td>
<td>162.6</td>
</tr>
<tr>
<td>112-90</td>
<td>66.9</td>
</tr>
<tr>
<td>B31</td>
<td>427.1</td>
</tr>
<tr>
<td>Mean</td>
<td>228.2</td>
</tr>
<tr>
<td>SEM</td>
<td>76.3</td>
</tr>
<tr>
<td><strong>RTN-treated cases</strong></td>
<td></td>
</tr>
<tr>
<td>112-94</td>
<td>24.7</td>
</tr>
<tr>
<td>WP241</td>
<td>0</td>
</tr>
<tr>
<td>WP178</td>
<td>12.4</td>
</tr>
<tr>
<td>6334</td>
<td>0</td>
</tr>
<tr>
<td>6335</td>
<td>0</td>
</tr>
<tr>
<td>Mean</td>
<td>7.4</td>
</tr>
<tr>
<td>SEM</td>
<td>5.5</td>
</tr>
</tbody>
</table>

*P<0.01, treated vs control.
hour.21-23 This window for reperfusion or recanalization may be much longer than once thought. A recent study by Young and coworkers16 compared the infarct volume of 6-hour MCAO with much longer than once thought. A recent study by Young and coworkers16 compared the infarct volume of 6-hour MCAO with that of much longer ischemic insults. They demonstrated that reperfusion, even after a 6-hour ischemic insult, can reduce the infarct volume by as much as 85%. Their findings, as well as those by Ringelstein et al,23 support the conclusion that early reperfusion, either by clot lysis or by rtPA, leads to better clinical outcome and smaller infarct size. In fact, when recanalization took > 8 hours, the eventual lesions invaded cortical MCA territories.24 Our studies strongly suggest that RTN technique can significantly reduce the time to reperfusion and thus maintain tissue viability until the ischemic tissue can be recanalized spontaneously or by tPA.

In future studies, more clinically relevant studies will address the ability of RTN to reduce or prevent stroke when applied several hours after the start of an ischemic insult. It is also presumed that in the future, RTN may be used to deliver important brain-protecting agents directly to the ischemic vascular bed. The addition of these agents theoretically could improve outcome and lengthen the window of opportunity during which a neurological deficit could be resolved.

Acknowledgments

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References


Editorial Comment

In the accompanying article, Frazee and colleagues showed that application of RTN baboons subjected to MCAO resulted in improved functional and pathological outcomes. Clearly, this technique supplies enough blood to the ischemic brain to prevent permanent neurological damage. It is important to consider what might be the potential clinical application of this technique in human stroke. Since the technique of necessity can be applied for only relatively short periods of time, it will be necessary to couple it with a more definitive treatment for the arterial obstruction, such as thrombolytic therapy. This raises 2 questions. First, assuming the patient arrives at a medical facility within the appropriate therapeutic window, would the combined application of RTN and thrombolytic therapy be more beneficial than thrombolytic therapy alone? A second important question is whether the application of RTN is safe under conditions of thrombolysis.

Clearly, additional work would be necessary before deciding that this technique is a useful addition to our armamentarium for treatment of human stroke.

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