Diffusion and Perfusion Magnetic Resonance Imaging Studies to Evaluate a Noncompetitive N-Methyl-d-aspartate Antagonist and Reperfusion in Experimental Stroke in Rats

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Background and Purpose: Diffusion magnetic resonance imaging (MRI) can quantitatively detect focal ischemic injury within minutes of onset, and perfusion MRI can evaluate the brain’s microcirculation. N-Methyl-d-aspartate (NMDA) antagonists and reperfusion can reduce lesion size in stroke models. We used diffusion and perfusion MRI to evaluate the in vivo effects of a noncompetitive NMDA antagonist, CNS 1102, in a temporary ischemia model.

Methods: Sixteen Sprague-Dawley rats underwent suture occlusion of the middle cerebral artery. Fifteen minutes after occlusion, animals were randomly assigned to treatment with CNS 1102 (n=10) or placebo (n=6), receiving a bolus of 1.1 mg/kg at that time and an infusion of 0.785 mg·kg⁻¹·h⁻¹ for the next 165 minutes. The placebo group received a saline bolus and infusion. Diffusion MRI studies by a spin-echo technique were initiated 30 minutes after occlusion and repeated every 30 minutes for the next 3 hours. Perfusion MRI studies were obtained using echo-planar imaging after injection of superparamagnetic iron oxide particles, immediately before and 15 minutes after withdrawal of the occluder at 3 hours after middle cerebral artery occlusion. At 24 hours, the animals were clinically evaluated (scale of 0 to 5) and electively killed, and the brain was stained with triphenyltetrazolium chloride to evaluate infarct size.

Results: Diffusion imaging demonstrated markedly reduced ischemic lesion area in the CNS 1102 group during occlusion—10.5±7.3% (mean±SEM) of the ischemic hemisphere (optic chiasm slice) at 30 minutes after occlusion versus 50.0±2.7% of the hemisphere in controls (P<.02). With reperfusion after 3 hours of temporary ischemia, diffusion imaging documented an additional 29% reduction of the ischemic lesion area in the CNS 1102–treated group (P<.01) compared with the prerereperfusion ischemic lesion area, with no change in the placebo group. During occlusion, perfusion imaging demonstrated a relative signal intensity decline of 31.5±7.7% in controls and 83.4±7.6% in the CNS 1102 group (P<.005), indicating better perfusion in the latter group. After removal of the occluder, perfusion improved in both groups and was not significantly different. Post mortem infarct volume was 53.8±20.0 mm³ in the CNS 1102 group and 216.8±16.1 mm³ in the controls (P<.0001). Clinical outcome at 24 hours was 1.1±0.4 in the CNS 1102 group and 4.0±0.5 (scale of 0 to 5) in the controls (P<.005).

Conclusions: This study demonstrates that CNS 1102 reduces early postischemic injury as documented by diffusion MRI and improves perfusion as documented by perfusion MRI and that reperfusion confers additional reduction of ischemic lesion size. (Stroke. 1993;24:2074-2081.)

Key Words • magnetic resonance imaging • N-methyl-d-aspartate • stroke, experimental

Receptor and diffusion of cerebral blood flow (CBF) with thrombolytic agents and cerebroprotection with N-methyl-d-aspartate (NMDA) receptor antagonists have great potential as therapies for acute ischemic stroke because both reperfusion and cytoprotective therapy reduce infarct size in animal stroke models.1-6 Although reperfusion after a brief duration of ischemia can salvage metabolically compromised brain tissues,7-10 recent studies suggest that there is a limited time window for successful reperfusion.3,11-13 Late reperfusion may result in pronounced brain edema, hemorrhagic transformation, and a worse outcome than with permanent vascular occlusion.3,7,14-17 Noncompetitive and competitive NMDA antagonists given after the onset of ischemia dramatically reduce brain injury in experimental stroke models.5,18-22 However, early termination of the drug therapy may result in delayed extension of cerebral infarction.22,23 Excessive and prolonged use of NMDA antagonists can cause adverse effects.24-26 A few studies have suggested that NMDA antagonists can ameliorate ischemic brain damage in animals with temporary arterial occlusion.27-29
These studies could not distinguish the effects of NMDA antagonists from those of reperfusion because of the inability to monitor evolution of ischemic brain damage in vivo before and after reperfusion.

Diffusion-weighted magnetic resonance imaging (DWI) can identify within minutes after the onset of experimental stroke areas of brain tissue that are ischemic and likely to progress to infarction.30-32 This technique has also recently been used within the first hours after stroke onset in humans.33 DWI measures the translational movement (Brownian motion or diffusion) of water in tissues.34-36 Energy deficits and the failure of ion pumps associated with ischemia cause accumulation of intracellular sodium and water (cytotoxic edema), which presumably reduces the diffusion coefficient of water in the ischemic tissue and results in hyperintensity on DWI.30 We previously demonstrated that DWI can quantitatively predict tissue destined for infarction within 30 minutes after occlusion of the middle cerebral artery (MCA) in rats.32 Initial DWI hyperintensity does not indicate irreversible damage because it can reverse completely or partially if CBF is restored within 2 hours after the onset of ischemia.31,37 We also have used this technique to assess pharmacologic intervention in a rat permanent MCA occlusion model and demonstrated the beneficial effects of a potent and selective noncompetitive NMDA antagonist, CNS 1102 [N-(1-naphthyl)-N'-(3-ethylphenyl)-N'-methyl-guanidine hydrochloride], on ischemic brain damage.22

In the present study, we used DWI, T2-weighted magnetic resonance imaging (T2WI) and dynamic contrast-enhanced perfusion magnetic resonance imaging (MRI) studies38,39 to assess the effects of postischemic administration of CNS 1102 on the evolution of brain injury during and after 3 hours of temporary ischemia in rats.

Materials and Methods

We studied 16 adult male Sprague-Dawley rats weighing 290 to 360 g. The experimental procedures were approved by the Animal Research Committee of the University of Massachusetts Medical School (#A-643). Animals were anesthetized with 400 mg/kg chloral hydrate given intraperitoneally. The rat’s body temperature was monitored with a rectal probe and maintained at 37.0°C with a heat lamp during the initial operation and with a water-circulating heating pad (K-module model K-20, American Pharmaseal, Valencia, Calif) during the MRI study. A polyethylene catheter was introduced into the left femoral artery for continuous monitoring of arterial blood pressure and to allow blood sampling for blood gas analysis at baseline, 30 minutes after occlusion, and 30 minutes after reperfusion. Another catheter was advanced into the vena cava through the left femoral vein for intravenous injection of CNS 1102 (Cambridge Neuroscience, Cambridge, Mass) or saline vehicle and for bolus intravenous injection of a superparamagnetic iron particle solution for perfusion MRI studies.

We induced the experimental ischemia by advancing a 4-0 monofilament nylon suture as a vascular occluder intracranially through the right common carotid artery (CCA) and into the proximal anterior cerebral artery, occluding the origin of the ipsilateral MCA.16,22,32,37 Restoration of vascular perfusion to the ischemic tissues was accomplished by pulling the occluder back to the CCA after 3 hours of MCA occlusion.37

Fifteen minutes after MCA occlusion, animals were randomly assigned to treatment with CNS 1102 (n=10) or saline vehicle (n=6). Drug-treated animals received an initial intravenous bolus of 1.13 mg/kg CNS 1102 in 0.3 mL of physiological saline followed by a continuous intravenous infusion of 0.785 mg·kg⁻¹·h⁻¹ of the drug in saline (1.0 mL/h) during the next 165 minutes. Maintenance doses of 3 mg/kg CNS 1102 in 0.265 mL of saline were administered intraperitoneally 5 and 9 hours after MCA occlusion. Animals in the vehicle control group received identical volumes of physiological saline without drug at the same time points as the drug-treated animals.

Animals were prepared for MRI studies after the MCA occlusion. Anesthesia was maintained with 0.5% to 1.0% isoflurane, and the animals’ heads were fixed to a “birdcage” radiofrequency coil. MRI studies were performed in a General Electric CSI-II 2.0-T/45-cm imaging spectrometer (GE Medical System, Fremont, Calif). The slice plane was standardized by first obtaining preview spin-echo images. DWI and T2WI studies were performed at 30-minute intervals beginning 30 minutes after occlusion over the next 150 minutes of MCA occlusion and then repeated 30 minutes after withdrawal of the occluder. Each DWI and T2WI scan consisted of four coronal slices of 2-mm thickness and 4-mm center-to-center separation over a 40-mm field-of-view (FOV); the optic chiasm (slice A) and the slice 4 mm caudal (slice B) were selected for analysis. Each image was acquired with two signal averages for each of 128 phase-encoding steps. The data were then zero-filled to give a final digital resolution of 256×256 points. Spin-echo DWI scans were collected over 8 minutes with a repetition time (TR) of 1800 milliseconds, an echo time (TE) of 45 milliseconds, half-sine-shaped diffusion-sensitive gradient pulses with duration of 10 milliseconds, pulse separation of 20 milliseconds, and strength of 15 G/cm, yielding a b value of 1,142 s/mm². Spin-echo T2WI scans were obtained over 10 minutes with TR of 2200 milliseconds and TE of 90 milliseconds.

Dynamic contrast-enhanced perfusion MRI studies were performed at slice A, using echo-planar imaging (EPI),40,41 immediately before and 15 minutes after withdrawal of the occluder. Sixteen EPI scans were acquired at 0.5-second intervals over an 8-second period immediately after a bolus injection of 0.3 mL of physiological saline containing 0.05 mmol superparamagnetic iron oxide particles per kilogram (AquaMag 100 magnetic fluid, Advanced Magnetics Inc, Cambridge, Mass) via an intravenous catheter over 1 second.39,42 The EPI scans were acquired using an incorporation of the EPI sequence in which the whole k-space is scanned in a “sawtooth” pattern.40,42 The FOV was 40 mm, and the digital resolution of the EP images was 64×64 points. The acquisition time of an individual EPI scan was 65.5 milliseconds.

After the MRI studies, the animals were allowed to recover from the anesthesia, and no obvious behavioral effects were seen in animals surviving for 24 hours. At 24 hours after occlusion, animals were evaluated neurologically using a six-point scale,22,37 which was modified from the scale used by Zea Longa et al:0, no
deficit; 1, failure to extend left forepaw fully; 2, circling to the left; 3, falling to the left; 4, no spontaneous walking with a depressed level of consciousness; and 5, dead. The animals were then anesthetized with an intraperitoneal injection of chloral hydrate (300 mg/kg) and decapitated. The brain was sectioned coronally at 2-mm intervals. Six sections containing the MCA territory were stained with a 2% solution of triphenyltetrazolium chloride (TTC) to identify infarcted tissue, fixed in formalin, and photographed.32,44

Data from DWI, T2WI, perfusion MRI, and TTC studies were blindly analyzed by an observer unaware of the animals’ experimental group. DWI and T2WI hyperintense regions in the ischemic hemisphere were determined by using a personal computer with image analysis software (OPTIMAS 3.0, Bioscan Products, Edmonds, Wash.). Gray-scale maps corresponding to actual signal intensity on a pixel-by-pixel basis were displayed on the computer monitor. Areas in the ischemic hemisphere were assigned as lesions when their gray levels appeared to a blinded observer to be greater than those in the contralateral hemisphere. The hyperintense area (lesion area) was divided by the ipsilateral hemispheric area to obtain the percent hemispheric lesion area (%HLA). Photographs of TTC-stained sections were evaluated with a computer-assisted digitizer (Sigma-Scan V3.10, Jandel Scientific, Corte Madera, Calif; and Numonics 2200, Numonics, Montgomeryville, Pa). The area not stained red with TTC was considered a lesion (an infarction) and was divided by the ipsilateral hemispheric area to determine %HLA. The infarct volume (mm³) was calculated by using a numerical integration of the lesion areas for all the TTC sections per animal and the distance between them.

In the perfusion MRI studies, the signal intensity within a region of interest (ROI) (1.0×1.0×2.0 mm³) in the upper sensorimotor cortex was bilaterally determined for each time point. The time from injection to the lowest signal intensity (time to nadir [TN]) and the maximum decline in signal intensity from the baseline (signal intensity decline [SID]) were determined for each time–signal intensity curve. The delay in TN (DTN) and the relative SID (RSID) in the ischemic ROI compared with those in the contralateral ROI were used to semiquantitatively assess the perfusion state. The perfusion state in the ipsilateral MCA territory was also qualitatively graded compared with the contralateral MCA territory (perfusion scale): 0, no difference; 1, delayed transit of the contrast agent; 2, an incomplete decline in signal intensity within a part of the MCA territory; 3, an incomplete decline in signal intensity within the entire MCA territory; and 4, no decline in signal intensity in the entire MCA area.37

For comparison of %HLA determined with DWI and TTC, a three-factor, repeated-measures analysis of variance (ANOVA) was performed, where a between-group factor was treatment (control and CNS 1102), and within-group factors were time of measurement (30 minutes, 3 hours, 3.5 hours, and 24 hours) and slices of measurement (slices A and B). T2WI %HLA before and after withdrawing the occluder was also analyzed with a three-factor, repeated-measures ANOVA. To compare physiological measurements and infarct volume, a two-factor, repeated-measures ANOVA and one-way ANOVA were used, respectively, depending on the numbers of factors and measurements in each animal. For comparison of the neurologic grading scale, DTN, RSID, and the perfusion scale between control and CNS 1102–treated animals, the Mann-Whitney U test was used. Changes in DTN, RSID, and the perfusion scale before and after withdrawal of occluder were assessed with the Wilcoxon signed-rank test. Linear and Spearman regression analyses were used for correlating parametric and nonparametric data sets, respectively. All values are mean ± SEM. A two-tail value of P<.05 was considered significant.

**Results**

Body weight, body temperature, blood pressure, and PO₂ were normal and not different between the groups during the observation period. Arterial PCO₂ was higher in treated rats than in control rats when the overall difference was tested (F1:14=6.50, P<.05). The difference was modest and not significant at baseline and 30 minutes after occlusion but was significant 30 minutes after withdrawal (32.3±1.4 mm Hg in CNS 1102–treated rats and 25.7±2.5 in controls; P<.05). Arterial pH was lower in the treated group than in the control group (F1:14=7.77, P<.02) and declined over time in both groups (F3:42=6.3, P<.005). Ad hoc analysis revealed a significant difference only at 30 minutes after withdrawal (7.30±0.01 in CNS 1102–treated rats and 7.36±0.01 in controls; P<.02). To assess the effects of CNS 1102 on brain temperature, brain and rectal temperatures were concomitantly evaluated in two rats subjected to 3 hours of temporary occlusion. The animals received the same dose of CNS 1102 as the animals in the MRI study. Temperature was maintained by a heating pad, but they did not undergo the MRI protocol. Brain temperature, as monitored by a thermocouple implanted stereotactically into the striatum of the occluded hemisphere, was 37°C just after occlusion and remained unchanged during the entire 165 minutes of CNS 1102 treatment as did the rectal temperature.

In all rats, perfusion MRI studies demonstrated hypoperfusion in the right MCA territory at 3 hours before withdrawing the occluder. In all animals, ROI analyses demonstrated a delay in the transit of the contrast agent on the occluded side. Values of RSID and the perfusion scale before withdrawal of the occluder were significantly better in CNS 1102–treated rats than in control rats, suggesting milder hypoperfusion in the former. After withdrawal of the occluder, the perfusion abnormalities improved significantly in both groups, indicating successful reperfusion (Table 1 and Fig 1).

At 30 minutes after occlusion, DWI revealed a large region of increased signal intensity in the ipsilateral MCA territory of all the control rats. These hyperintense lesions remained unchanged in size over the next 2.5 hours of occlusion and 30 minutes after withdrawal of the occluder. DWI studies at 30 minutes after occlusion showed that 7 of 10 rats treated with CNS 1102 had no detectable abnormality, 2 had lesions confined to the caudoputamen or a small cortical region, and 1 had an extensive lesion comparable to those detected in control rats. By 3 hours after occlusion, hyperintense areas were seen in 7 rats. After withdrawal of the occluder, the hyperintensity resolved completely in 1 of these rats.
TABLE 1. Perfusion Magnetic Resonance Imaging Studies

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Time</th>
<th>Control (n=6)</th>
<th>CNS 1102 (n=10)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTN, s</td>
<td>Prewithdrawal</td>
<td>1.0 (0.5-1.5)</td>
<td>0.75 (0-1.5)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Postwithdrawal</td>
<td>0.25 (0-1.0)</td>
<td>0 (0-1.0)†</td>
<td>NS</td>
</tr>
<tr>
<td>RSID, %</td>
<td>Prewithdrawal</td>
<td>31.5±7.7</td>
<td>83.4±7.6</td>
<td>&lt;.005</td>
</tr>
<tr>
<td></td>
<td>Postwithdrawal</td>
<td>70.3±15.7†</td>
<td>97.9±3.3†</td>
<td>NS</td>
</tr>
<tr>
<td>Perfusion scale</td>
<td>Prewithdrawal</td>
<td>4 (3-4)</td>
<td>2.5 (1-3)</td>
<td>&lt;.005</td>
</tr>
<tr>
<td></td>
<td>Postwithdrawal</td>
<td>0 (0-3)†</td>
<td>0 (0-2)†</td>
<td>NS</td>
</tr>
</tbody>
</table>

DTN indicates delay in time to nadir; and RSID, relative signal intensity decline. Values are expressed as median values (range) for DTN and perfusion scale and as mean±SEM for RSID.

*P values of difference between the control and CNS 1102–treated rats by Mann-Whitney U test.
†P<.05, ‡P<.005 different from the prewithdrawal values by Wilcoxon signed-rank test.

decreased in size in 4, and remained unchanged in 2 (Fig 2).

T2WI studies detected no abnormalities at 30 minutes after occlusion. At 3 hours, before withdrawal, T2WI could not detect ischemic lesions or underestimated the size of the ischemic lesion by approximately half compared with the DWI studies. After withdrawal of the occluder, the T2WI lesion size remained unchanged in drug-treated rats but increased in control rats.

Three control but no treated rats died with massive brain edema and brain herniation before 24 hours. The neurological scale at 24 hours was 1.1±0.4 in the treated group, significantly better than the score in the control group (4.0±0.5; P<.005). Post mortem TTC studies demonstrated that all control rats had an extensive cerebral infarction encompassing almost the entire MCA territory and that the %HLA at slices A and B were significantly smaller in the CNS 1102 group (Table 2). Three rats treated with CNS 1102 had no measurable infarction, 2 had a caudoputaminal infarction alone, 1 had a small cortical infarction alone, and the remaining 4 rats had both cortical and caudoputaminal infarctions. Total infarct volume in rats treated with CNS

Fig 1. Region-of-interest analyses for representative perfusion magnetic resonance imaging studies, showing changes in signal intensities over time immediately after injection of iron particle solution. Studies at 3 hours (prewithdrawal) and immediately after withdrawal of a vascular occluder in a rat treated with CNS 1102 (A and B) and that with saline vehicle (C and D). Withdrawal of the occluder from the right side improved delay in time to nadir (DTN) and relative signal intensity decline (RSID) in both groups. Values in RSID were lower before and after withdrawal in the saline control rat than in the CNS 1102–treated rat.
1102 was 53.8±20.0 mm³, significantly smaller than in control rats (216.8±16.1 mm³, P<.0001). In vivo DWI studies demonstrated that treatment with CNS 1102 significantly reduced %HLA at slices A and B (F,14=17.43, P<.01; Table 2). The lesion sizes were not the same over time (F,4,14=7.31, P<.0001) and were different between slice A and B (F,1,14=15.43, P<.02). No significant interactions were observed among the factors. The DWI hyperintense areas obtained just before reperfusion (3 hours after occlusion) in the CNS 1102–treated group decreased significantly in size by 29% (average reduction rate at slices A and B, P<.01) after withdrawal of the occluder (Fig 2) but not in the control group. In either group, postwithdrawal DWI studies accurately predicted TTC lesion size and location. TTC %HLA was significantly correlated with postwithdrawal DWI %HLA (y=0.91x+4.0, n=32, r=.921, P<.0001). T2WI lesion sizes at 3 hours after occlusion, just before withdrawal, and 3.5 hours after occlusion (30 minutes after withdrawal) were significantly smaller in the treated group than in the control group (F,1,14=5.26, P<.05; Table 3).

**Discussion**

The present study demonstrated that the noncompetitive NMDA antagonist CNS 1102 is cerebroprotective in a model of transient focal ischemia in rats. Administration of CNS 1102 beginning 15 minutes after MCA occlusion reduced the brain damage caused by 3 hours of temporary MCA occlusion, as measured by in vivo DWI, neurological evaluation, and post mortem histology. T2WI studies also detected the beneficial effects of CNS 1102, but not until late in the occlusion period, and almost always underestimated the size of the lesion. Neurological outcome in rats evaluated 24 hours after the onset of ischemia was significantly better in the CNS 1102–treated group than in the saline-treated control group. Higher pH and lower Pco₂ in the saline-treated

**TABLE 2. Percent Hemispheric Lesion Area at Slices A and B Determined With DWI at 30 Minutes, 3 Hours (Prewithdrawal), and 3.5 Hours (Postwithdrawal) and by TTC Staining at 24 Hours**

<table>
<thead>
<tr>
<th>Time</th>
<th>Slice</th>
<th>Control (n=6)</th>
<th>CNS 1102 (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 Minutes</td>
<td>A</td>
<td>50.0±2.7</td>
<td>10.5±7.3</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>22.3±7.6</td>
<td>6.1±4.6</td>
</tr>
<tr>
<td>3 Hours</td>
<td>A</td>
<td>56.8±3.9</td>
<td>25.5±10.4</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>41.3±4.4</td>
<td>16.1±5.3</td>
</tr>
<tr>
<td>3.5 Hours</td>
<td>A</td>
<td>61.6±8.2</td>
<td>17.2±7.6</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>33.7±5.0</td>
<td>12.1±4.8</td>
</tr>
<tr>
<td>24 Hours</td>
<td>A</td>
<td>62.8±3.3</td>
<td>18.2±6.5</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>42.5±7.8</td>
<td>9.8±3.9</td>
</tr>
</tbody>
</table>

DWI indicates diffusion-weighted magnetic resonance imaging; and TTC, triphenyltetrazolium chloride.

Three-factor, repeated-measure ANOVA: F,1,14=17.342 for treatment (P<.001), F,3,42=7.306 for time (P<.001), F,1,14=15.433 for slice (P<.002), F,3,42=1.44 for interaction between treatment and time (P=NS), F,1,14=5.21 for interaction between treatment and slice (P=NS), F,3,42=0.345 for interaction between time and slice (P=NS), and F,3,42=1.754 for interaction among treatment, time, and slice (P=NS).

**TABLE 3. Percent Hemispheric Lesion Area at Slices A and B Determined With T2-Weighted Magnetic Resonance Imaging at 3 Hours (Prewithdrawal) and 3.5 Hours (Postwithdrawal)**

<table>
<thead>
<tr>
<th>Time</th>
<th>Slice</th>
<th>Control (n=6)</th>
<th>CNS 1102 (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Hours</td>
<td>A</td>
<td>28.8±8.2</td>
<td>13.8±6.3</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>10.4±7.0</td>
<td>6.5±3.7</td>
</tr>
<tr>
<td>3.5 Hours</td>
<td>A</td>
<td>42.1±5.4</td>
<td>14.0±5.9</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>17.4±5.8</td>
<td>4.8±2.4</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM.

Three-factor, repeated-measure ANOVA: F,1,14=5.257 for treatment (P<.05), F,3,42=5.365 for time (P=NS), F,1,14=36.5 for slice (P<.0001), F,1,14=1.868 for the interaction between treatment and time (P=NS), F,1,14=7.237 for the interaction between treatment and slice (P<.02), F,1,14=2.124 for the interaction between time and slice (P=NS), and F,1,14=0.059 for the interaction among treatment, time, and slice (P=NS).
raths than in the CNS 1102–treated rats were likely attributable to spontaneous hyperventilation induced by more-pronounced brain damage in the former. Brain temperature could not be monitored in the MRI unit because of the high-field-strength magnet, but CNS 1102 did not lower brain temperature in the occluded hemisphere when it was directly measured in a small group of animals that did not undergo the MRI protocol.

We have previously reported that CNS 1102 confers similar protection against ischemic damage after permanent MCA occlusion in rats.22 In that study, however, animals treated with CNS 1102 tended to have cerebral infarcts at 24 hours that were larger than the size predicted with DWI studies performed 3 hours after occlusion, suggesting that the ischemic injury worsened over the next 21 hours. The short plasma-elimination half-life of CNS 1102 in rats (63 minutes) and the limited duration of treatment (3 hours) likely contributed to this result. The present investigation used exactly the same drug treatment protocol as in our earlier study but restricted the duration of MCA occlusion to 3 hours. We observed that in animals treated with CNS 1102, the ischemic area identified by DWI studies actually diminished in size by 29% after reperfusion. Moreover, the size and position of infaracts measured histologically at 24 hours were almost identical to those of lesions identified by DWI studies 30 minutes after reperfusion. We conclude that reperfusion prevents the delayed increase in ischemic damage previously observed and that NMDA antagonist treatment combined with reperfusion enhances rescue of ischemic tissue after focal cerebral ischemia in rats.

Several laboratories, including ours, have studied temporary MCA occlusion in rats. In general, reperfusion must begin within 2 hours after the onset of ischemia to provide benefit.10,13,15–17,37,40 Longer durations of ischemia produce at least as much damage as does permanent occlusion. We previously observed that the size of the DWI hyperintense areas declined significantly (55% decline) when blood flow was reestablished after 1 hour of MCA occlusion but not when reperfusion began after 2 hours.37 We concluded that reperfusion after 1 hour but not 2 hours of ischemia can salvage compromised tissue that would otherwise progress to infarction. In the present study, we demonstrated that early treatment with an NMDA antagonist can extend to at least 3 hours the period during which reperfusion is beneficial.

Although the present experiments were not specifically designed to address the issue, our results provide indirect evidence that delayed reperfusion may actually exacerbate ischemic neuronal damage. In saline-treated animals, 3 hours of MCA occlusion followed by reperfusion resulted in extensive cerebral infarctions with prominent brain edema. Animal deaths in this group (50%) were also more frequent than we observed in rats after permanent MCA occlusion (9.5%, unpublished observation) or 1 to 2 hours of temporary occlusion (10%).37 Tissue abnormalities detected by T2WI studies expanded after withdrawal of the occluder in saline treated animals. The T2WI abnormalities after brain ischemia have been associated with the development of vasogenic edema, although there is conflicting evidence.45,46

We used perfusion MRI studies to document hypoperfusion during arterial occlusion and to verify reperfusion after withdrawing the occluder. The perfusion MRI studies can provide quantitative data such as mean transit time, cerebral blood volume, and CBF, which is equal to the ratio of cerebral blood volume to the mean transit time, if intracapillary concentration of a contrast medium is calculated from signal intensities, the arterial input function is measured, and the γ-fitting approach is applied to time-concentration curves.38,39,41 Although such quantitative data could not be obtained in the present study, the semiquantitative and qualitative evaluations strongly suggested that vascular perfusion and, by inference, CBF during MCA occlusion were better in CNS 1102–treated rats than in saline-treated rats.

Although NMDA antagonists are not thought to have direct vasomotor actions, there is conflicting evidence concerning their effects on cerebral circulation. Park et al40 observed that MK-801 did not change the extent of the ischemic areas (defined as CBF less than 30 mL·100 g−1·min−1) measured 40 minutes after MCA occlusion in deeply anesthetized and mechanically ventilated rats. They concluded that the anti-ischemic effects of MK-801 that they observed under the same experimental conditions5 could not be attributed to improvement of CBF. Likewise, Deszi et al41 found that MK-801 did not alter CBF at the periphery of the MCA territory after experimental stroke in cats.

On the other hand, MK-801 has been reported to alter regional CBF in unanesthetized animals,46,49 and several recent studies have raised the possibilities that NMDA antagonists may improve CBF during arterial occlusion.29,49,50 A competitive NMDA antagonist, CGS-19755, increased CBF in both ischemic and nonischemic hemispheres 4 hours after arterial occlusion in rats.51 In that study, CBF was measured after the animals recovered from anesthesia. Buchan et al42 observed that MK-801 significantly increased blood flow to ischemic brain in lightly anesthetized normotensive rats but not in spontaneously hypertensive rats, suggesting that the cerebralprotective effects of NMDA antagonists may result in part from improvements in CBF. Alternatively, by preventing cellular injury, NMDA antagonists may facilitate improvement of collateral circulation. In any case, effects on CBF appear to vary depending on the animal model being studied and, especially, the degree of anesthesia at the time CBF is measured.

In conclusion, intervention with CNS 1102 beginning shortly after the onset of ischemia prolonged the time window for effective reperfusion and possibly mitigated adverse effects resulting from late reperfusion. Successful restoration of blood flow, in turn, salvaged additional tissue. DWI offers a rapid and noninvasive way to monitor the effectiveness of both therapeutic strategies and should be adaptable to clinical studies of human stroke patients.

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Diffusion-weighted magnetic resonance imaging (DWI) can be used to produce images with contrast dependent on the translational movement of water. Furthermore, these techniques can be expanded to allow the quantitation of the apparent diffusion coefficient of water ($AD_{Cw}$). In cerebral ischemia, it has been previously demonstrated that the $AD_{Cw}$ declines by up to a factor of 2 after the onset of ischemia. This decline is rapid, preceding changes in any other $^1$H magnetic resonance imaging parameter studied to date and represents a decrease in the translational movement of water. An important question to address is whether the information derived from these noninvasive measurements can be used to determine the pathophysiological status of ischemic brain damage.

In the preceding article, Minematsu and colleagues have addressed the question of whether DWI can be used to document the therapeutic use of a noncompetitive N-methyl-D-aspartate antagonist in cerebral ischemia. Indeed, this group demonstrated that the markedly reduced ischemic lesion area seen with DWI for the treated group (both during ischemia and after reperfusion) was confirmed by a reduced infarct volume assessed histologically (TTC staining). Additionally, clinical outcome of the treated group at 24 hours was significantly better than in controls.

Although there is considerable interest in assessing the changes in $AD_{Cw}$, seen after cerebral ischemia, the specific mechanism(s) for these changes are unknown at this time. Nevertheless, the significance of the approach presented in this article lies in the possibility of developing a clinically relevant noninvasive method for staging the severity, extent, and prognosis of stroke-induced brain damage in humans.

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Diffusion and perfusion magnetic resonance imaging studies to evaluate a noncompetitive 
N-methyl-D-aspartate antagonist and reperfusion in experimental stroke in rats.
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