Neuroprotection by Excitatory Amino Acid Antagonist Augments the Benefit of Thrombolysis in Embolic Stroke in Rats

Tomas Sereghy, MD; Karsten Overgaard, MD; Gudrun Boysen, MD, DMSc

Background and Purpose: The effects of delayed thrombolysis with alteplase and neuroprotection with an excitatory amino acid receptor antagonist and their combination were tested in an embolic stroke model.

Methods: In 61 rats the carotid artery territory was embolized with arterial-like fibrin-rich clots. Hemisphere cerebral blood flow before and after embolization was measured by intraarterial 133Xe injection method. The animals were assigned to one of the following treatments: (1) vehicle-treated controls (n=15); (2) dizocilpine 1 mg/kg IV 5 minutes after embolization (n=16); (3) alteplase 20 mg/kg as an intravenous continuous infusion starting 2 hours after embolization (n=16); and (4) both agents (n=14). Carotid angiography displayed the site of occlusion of the cerebral arterial tree immediately after and 3 hours after embolization, and the clinical neurological score was assessed after the rats recovered from anesthesia and before the rats were killed. Brains were fixed after 2 days and evaluated neuropathologically; infarct volume affecting cortical and deep brain structures was measured separately.

Results: Both alteplase and dizocilpine reduced the total infarct volume (*P<.05 and *P<.04, respectively, Mann-Whitney tests). Dizocilpine reduced the incidence of cortical infarctions by 48% (*P<.001, Fisher’s test). Only the combined treatment significantly reduced deep brain infarctions (*P<.03, Mann-Whitney test). The combined treatment also improved the clinical score by 83% compared with controls, by 75% compared with the group treated by dizocilpine alone, and by 50% compared with the group treated by alteplase alone. Sixty-seven percent of thrombolytic-treated animals recanalized completely compared with 39% of those given no thrombolytics (*P<.05, Fisher’s test). The clinical outcome correlated with infarct size (*P<.01, Spearman test).

Conclusions: Our results document comparable efficacy of delayed thrombolysis and excitatory amino acid receptor antagonism in this model and suggest that combination of these two therapeutic approaches may yield additional benefit in treatment of thromboembolic stroke, particularly in cases where deep brain (end-artery-supplied) structures are affected. (Stroke. 1993;24:1702-1708.)

KEY WORDS • cerebral ischemia • N-methyl-D-aspartate • thrombolytic therapy • rats

The majority of ischemic strokes are caused by acute occlusion of cerebral arteries. One strategy for stroke treatment is restoration of blood flow within the ischemic area by thrombolytic agents. Promising results of pilot trials on thrombolysis in both carotid and vertebrobasilar artery thrombosis have been published recently. These reports as well as reports of thrombolysis in animal stroke models suggest the need for urgent administration of thrombolytics to gain maximal benefit and minimize the risk of bleeding complications. Glutamate and aspartate are released in large amounts during cerebral ischemia and in trace amounts during cortical spreading depression. One of the suggested pathophysiological pathways of ischemic excitotoxic neuronal injury is calcium influx via the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor–gated channel.

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A number of experiments have shown reduction of cortical infarct size and improvement of neurological clinical outcome or survival with various NMDA receptor antagonists administered after induction of focal cerebral ischemia. Other than their specificity as ligands, the most limiting factor for competitive NMDA antagonists is currently their hydrophilicity, which causes low and slow passage across the blood-brain barrier. This would play a minor role if the benefit of excitatory amino acid (EAA) receptor antagonists is due to their action during reperfusion of areas suffering from a period of total ischemia where the blood-brain barrier is severely damaged, but it would be of great importance if they act on low-flow areas surrounding the ischemic core, where the blood-brain barrier is completely or partially preserved. Therefore, we have chosen for our experiment dizocilpine maleate as a potent NMDA antagonist with an excellent blood-brain partition coefficient.

The aim of this study was to explore the possible benefit of the combination of both thrombolytic and neuroprotective therapeutic strategies in a well-established model of ischemic stroke.
Materials and Methods
In 61 male Sprague-Dawley rats weighing 300 to 400 g, anesthesia was induced with diazepam intraperitoneally and fluanison and fentanyl subcutaneously and prolonged with supplementary doses when necessary, as described previously.33 The body temperature was servo-controlled at 37 ± 0.5°C by rectal temperature monitoring and heating lamp. Mean arterial blood pressure (MABP), PaCO₂, PaO₂, and pH were repeatedly measured (Radiometer ABL 2, Copenhagen, Denmark) through a femoral arterial line.

Preparation of the Emboli
Arterial rat blood was aspirated into a PP 50 (internal diameter, 0.58 mm) polyethylene tube and left for 2 hours clotting at room temperature and for 22 hours at 4°C. Before embolization, 50 mm of the tube containing a retracted clot was cut off and attached in the middle of a system built of two syringes interconnected by a 30-cm PP 50 tube filled with saline. The clot was moved repeatedly back and forth within the tube by alternately compressing the syringes for 5 minutes. By this method thread-shaped fibrin-rich clot is obtained.16

Operative Procedure
The right external carotid artery and its branches were exposed, and the pterygopalatine, thyroid, and occipital arteries were ligated. A polyethylene PP 25 catheter was inserted through a transverse arteriotomy of the external carotid artery with the tip 2 mm distal to the bifurcation, fixed with ligatures, and kept patent by continuous flow of heparinized (5 IU/mL) saline. The total amount of heparin did not exceed 15 IU per animal. Care was taken not to injure the intima.

Embolization and Cerebral Blood Flow Measurements
Just before and after embolization, cerebral blood flow (CBF) was measured using an intracarotid bolus injection of 0.15 to 0.20 mL saline containing 133Xe (5 to 10 mCi/mL, Amersham). As previously described,33 the preembolic clearance was recorded as the initial 15-second slope. Then the common carotid artery was occluded temporarily by gentle lifting of a thread placed around the artery 15 mm proximal to the bifurcation, and the clot was injected into the carotid catheter. In the following 15 seconds, the postembolic 133Xe efflux was recorded.

Angiography
Immediately after and 3 hours after embolization, angiography was performed via the carotid catheter by bolus injection of 0.2 mL heparinized (5 U/mL) iohexol (Omnipaque, 300 mg iodine per milliliter; Nycomed, Denmark). Technical data have been reported earlier.33 Angiograms were evaluated blindly according to the score: 0, patent arteries (Fig 1, left); 1, middle cerebral artery (MCA) branch occlusion; 2, MCA stem occlusion (Fig 1, right); 3, internal carotid artery occlusion. The animals with less than 50% CBF reduction and no visible occlusion on the first angiography were excluded from the study.

Treatment Regimens
The animals were assigned to one of the following treatments: (1) vehicle only (control group); (2) dizzclplin hydrogen maleate (MK-801) (RBI, Mass) 1 mg/kg IV 5 minutes after embolization (MK-801 group); (3) alteplase (Actilyse, Boehringer Ingelheim Pharmaceuticals, Inc) 20 mg/kg as a continuous infusion starting 2 hours after embolization and distributed over 45 minutes through a femoral venous line (alteplase group); and (4) combined treatment with both agents as described above (combined group).

After the second angiography, femoral and neck wounds were closed after ligation of vessels. The anesthesia was reverted with naloxone (Narcanti, Du Pont Pharmaceuticals) 0.1 mg IM. After recovering from anesthesia and just before being killed, the neurological deficit was assessed using the rating scale described by Bederson et al.34 Rats were held by the tail 1 m above the floor. Normal rats extended both forelimbs toward the floor and were assigned grade 0. Rats with flexion of the forelimb contralateral to the injured hemisphere were graded 1, rats with reduced resistance to lateral push toward the paretic side were graded 2, and rats with spontaneous circling toward the paretic side were graded 3. Animals dying before being killed were assigned grade 3 on the second clinical evaluation.

Neuropathology
After 2 days' survival the animals were killed by formalin perfusion fixation and decapitated, and the heads were kept 24 hours embedded in 40% formalin solution. The brains were harvested, embedded in formalin for 3 weeks, and dehydrated. Horizontal serial sections of 4-μm slice thickness with a distance of 0.4 mm between each were stained with hematoxylin-eosin. Infarcted areas were characterized by nuclear pyknosis and cytoplasmic eosinophilia, swelling, and vacuolization of neurons. Infarcts affecting one of two following brain regions (Fig 2, top), (1) neocortex and entorhinal cortex as “cortical infarction” and (2) basal ganglia, thalamus, hippocampus, amygdala, and brain stem as “deep brain structure infarctions,” were delineated separately under microscope by drawing pen without knowledge of the treatment regimen. The total area is the sum of both aforementioned areas. Bulbous olfacto-rus and cerebellar structures were not included in our measurements. Affected hemisphere and infarct volumes were calculated as areas multiplied with the distance between sections using a flat-bed scanner and digital image analyzing program (Sidney Data, Copenhagen, Denmark).

Calculations and Statistical Analyses
All percentages were calculated as the following ratio: 100 × (value 1 − value 2)/value 1. Nonparametric statistical analyses (Mann-Whitney U test for unpaired observations, Wilcoxon matched-pairs test for paired observations, Spearman test for correlation of ranked pairs, and Fisher’s exact probability test for binomial data) of our data were performed because our data sets were not normally distributed.
Results

The physiological parameters are displayed in Table 1. No differences were found in any parameters measured at the time of embolization. At the end of the operating procedure, all treated groups had significantly lower pH, and both MK-801–treated groups had significantly lower MABP than controls.

The main results of the study are shown in Table 2. The MK-801 group had an unintentional but significantly higher CBF reduction than the control group. No differences in initial angiographic occlusion values were found among any groups. The animals were sedated as they recovered from anesthesia; most showed flexion of the forelimb contralateral to the site of brain damage, asymmetrical posture, tilting of the head, and circling. All except two animals obtained the highest grade on the postoperative clinical scoring. The two animals without postoperative clinical damage (one treated by alteplase and one by both agents) developed no infarction. Three control animals died before being killed. They all had large infarctions without hemorrhage. The basal ganglia were affected in most cases, followed by the MCA cortical area and the thalamic and stem structures.

Effect of Thrombolysis

Comparing the alteplase group with the control group, thrombolysis reduced the total infarct size by 80% ($P=.05$). The reductions of separate cortical and deep brain infarctions were not significant. Clinical outcome was improved by 67% on the 4-point scale ($P=.03$). Comparing the MK-801 group with the combined group, alteplase yielded reduction of total infarct size by 87% ($P=.09$) and reduction of clinical damage by 75% ($P=.13$, all Mann-Whitney tests). Comparing the first and second angiograms of each animal, there was significant recanalization during the 3-hour period in all groups (Wilcoxon test). Twenty of 30 animals treated with alteplase recanalized completely (angiographic score value, 0), while this was the case for only 12 of 31 given no alteplase (therapeutic gain, 28%; $P=.05$, Fisher’s exact test). The 10 completely recanalized animals from the alteplase group developed smaller infarctions (median, 0.39% of hemisphere volume) than the 6 nonrecanalized or partially recanalized animals (median, 8.72%; $P=.05$, Mann-Whitney test). Comparing the postoperative and the preanesthesia clinical score of each animal, the thrombolytic-treated animals recovered better than controls ($P=.005$ for both groups, whereas this clinical recovery reached only a 5% significance level in controls, all Wilcoxon tests).

Effect of NMDA Blockade

Infarct size reductions by MK-801 and their significance are displayed in Table 2. Addition of MK-801 to alteplase yielded significant reduction of cortical infarctions only. Only three animals of each MK-801–treated group developed cortical infarction (Fig 2). Comparing

Fig 1. Left, Angiogram shows patent hemispheric arterial supply, with angiographic score value of 0. The following arteries are identified: internal carotid artery (thick arrow), middle cerebral artery (large thin arrow), and middle cerebral artery branches (small thin arrows). Right, Angiogram shows occlusion (complete filling defect) of middle cerebral artery (angiographic score value of 2) with enhanced filling of posterior cerebral artery.
the incidence of cortical infarctions, the therapeutic gain was 55% between the controls and the MK-801 group (P<.01) and 41% between the alteplase group and the combined group (P=.05, both Fisher's tests). MK-801 alone (P=.003) as well as in addition to alteplase (P=.005) improved the clinical recovery rate (compared with P=.05 in controls, Wilcoxon tests).

Among controls, the value of occlusion on the post-treatment angiography correlated with the preeuthanasia clinical score (r=.53, P=.04) as well as the CBF reduction with the deep brain infarctions (r=.56, P=.05). Those correlations were poor among pooled treated animals (r=.07 and r=.2, respectively). Among the MK-801–treated animals, the preeuthanasia clinical score correlated with the deep brain (r=.53, P=.04) and total infarction values (r=.52, P=.04) but not with the cortical values (r=−.2). There was correlation between total infarction volume and preeuthanasia clinical score among all animals (r=.37, P=.004, all Spearman tests). Eleven completely or partially recanalized control animals (patent arteries or MCA branch occlusion only on the second angiogram) developed smaller cortical infarctions than four nonrecanalized controls (P=.05, Mann-Whitney test).

**Discussion**

While some investigators have found benefit of NMDA antagonists independent of regional CBF (rCBF) within no- or low-flow areas, others have shown an increase in rCBF after MK-801 administration. McCulloch and associates used [14C]iodoantipyrine and deoxyglucose autoradiography to assess the rCBF changes and local glucose utilization in both conscious and halothane-anesthetized rats after NMDA.
antagonism. They found an increased rCBF in the neocortex, caudate nucleus, entorhinal cortex, and corpus callosum of conscious rats; glucose utilization was increased in the limbic structures, unaltered in the basal ganglia, and downregulated in the neocortex. Whether those changes in local CBF and metabolism can be caused by dopamine receptor blockade by MK-801, by NMDA antagonism alone (thereby allowing activation of local metabolism of adrenergic monoamines), or by some other interaction remains to be clarified. The NMDA receptor is blocked by acidosis, which could explain the lack of benefit when it is used in models of global severe ischemia.

The objection has been raised that in some experiments documenting the benefit of NMDA antagonism the outcome could be attributed to hypothermia during and after the ischemic period rather than to its synaptic blockade. In our experimental setting, the animals were maintained normothermic for at least 5 hours after embolization, after which period hypothermia might have occurred, but it would probably have been without pathophysiological importance. In our model, in which only small infarction (median, 20% of hemisphere volume in controls) develops and the brain surface is not exposed, the brain temperature corresponds well with the body temperature, so that rectal temperature monitoring is satisfactory. Hypotension in the two dizocilpine-treated groups is consistent with reports of others. This finding, as well as the acidosis that appeared in all our treated groups, could not improve the outcome.

There was a high variance of infarction volumes in the control group, and this variance further increased with every possibly beneficial intervention that was examined. This was caused partially by the variance of the location of the postembolic occlusion with our method of embolization and partially by the fact that the tendency to spontaneous recanalization occurs in most animals even without thrombolytic treatment (Table 2). The variance of infarctions that developed makes the statistical analysis difficult and requires nonparametric statistical analysis, but it also resembles the clinical situation in the stroke population. To avoid further increases in the variance of this end point by having small infarctions or no infarctions caused by insufficient embolization in the control group, it was necessary to use the first angiogram in combination with CBF decrease as exclusion criteria in our experimental design, as reported in the “Materials and Methods” section.

There is consistent evidence that unlike the cortex, systemic NMDA blockade does not protect basal ganglia

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Table 1. Physiological Parameters in 61 Rats in an Embolic Stroke Model

<table>
<thead>
<tr>
<th>Group</th>
<th>MABP, mm Hg</th>
<th>pH</th>
<th>P02, mm Hg</th>
<th>O2 Saturation</th>
<th>PCO2, mm Hg</th>
<th>MABP, mm Hg</th>
<th>pH</th>
<th>P02, mm Hg</th>
<th>O2 Saturation</th>
<th>PCO2, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>85.0</td>
<td>7.34</td>
<td>80.1</td>
<td>0.93</td>
<td>50.5</td>
<td>89.0</td>
<td>7.38</td>
<td>72.0</td>
<td>0.92</td>
<td>44.4</td>
</tr>
<tr>
<td>(n=15)</td>
<td>(84.0-94.0)</td>
<td>(7.32-7.35)</td>
<td>(71.0-85.4)</td>
<td>(0.92-0.94)</td>
<td>(48.1-53.2)</td>
<td>(80.0-96.0)</td>
<td>(7.36-7.40)</td>
<td>(68.1-76.5)</td>
<td>(0.91-0.93)</td>
<td>(38.8-49.4)</td>
</tr>
<tr>
<td>MK-801</td>
<td>82.0</td>
<td>7.33</td>
<td>79.4</td>
<td>0.93</td>
<td>53.3</td>
<td>85.5</td>
<td>7.35</td>
<td>67.4</td>
<td>0.91</td>
<td>49.1</td>
</tr>
<tr>
<td>(n=16)</td>
<td>(78.5-88.0)</td>
<td>(7.31-7.34)</td>
<td>(76.2-82.8)</td>
<td>(0.92-0.94)</td>
<td>(50.6-54.1)</td>
<td>(80.5-97.5)</td>
<td>(7.33-7.37)</td>
<td>(56.7-75.6)</td>
<td>(0.86-0.93)</td>
<td>(44.6-51.4)</td>
</tr>
<tr>
<td>Alteplase</td>
<td>89.0</td>
<td>7.34</td>
<td>79.8</td>
<td>0.93</td>
<td>51.9</td>
<td>77.5</td>
<td>7.35</td>
<td>72.7</td>
<td>0.92</td>
<td>42.8</td>
</tr>
<tr>
<td>(n=16)</td>
<td>(86.5-91.5)</td>
<td>(7.31-7.36)</td>
<td>(74.5-86.9)</td>
<td>(0.92-0.95)</td>
<td>(46.3-56.4)</td>
<td>(66.0-83.5)</td>
<td>(7.31-7.37)</td>
<td>(69.2-78.7)</td>
<td>(0.91-0.93)</td>
<td>(40.1-48.3)</td>
</tr>
<tr>
<td>Combined</td>
<td>81.0</td>
<td>7.35</td>
<td>85.0</td>
<td>0.94</td>
<td>50.8</td>
<td>73.0</td>
<td>7.34</td>
<td>77.1</td>
<td>0.93</td>
<td>44.0</td>
</tr>
<tr>
<td>(n=14)</td>
<td>(70.0-84.0)</td>
<td>(7.33-7.38)</td>
<td>(75.9-88.3)</td>
<td>(0.92-0.95)</td>
<td>(47.2-52.0)</td>
<td>(71.0-78.0)</td>
<td>(7.32-7.36)</td>
<td>(88.9-89.0)</td>
<td>(0.91-0.95)</td>
<td>(41.7-45.9)</td>
</tr>
</tbody>
</table>

Data are median values with interquartile intervals in parentheses. MABP indicates mean arterial blood pressure.

Table 2. Main Results of a Combination of MK-801 and Alteplase in an Embolic Stroke Model

<table>
<thead>
<tr>
<th>Group</th>
<th>CBF Reduction, %</th>
<th>Immediately After Embolization</th>
<th>3 Hours After Embolization</th>
<th>Postoperative</th>
<th>Preeuthanasia</th>
<th>Deep Brain Structures</th>
<th>Cortex</th>
<th>Total Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>57.0</td>
<td>2.0</td>
<td>1.0</td>
<td>3.0</td>
<td>3.0</td>
<td>13.2</td>
<td>9.0</td>
<td>19.6</td>
</tr>
<tr>
<td>(n=15)</td>
<td>(36.0-63.0)</td>
<td>(2.0-3.0)</td>
<td>(0.0-1.0)</td>
<td>(3.0-3.0)</td>
<td>(1.0-3.0)</td>
<td>(1.2-16.9)</td>
<td>(0.0-17.5)</td>
<td>(3.9-37.4)</td>
</tr>
<tr>
<td>MK-801</td>
<td>76.0</td>
<td>2.0</td>
<td>0.0</td>
<td>3.0</td>
<td>2.0</td>
<td>4.6</td>
<td>0.0†</td>
<td>4.5*</td>
</tr>
<tr>
<td>(n=16)</td>
<td>(56.0-80.5)</td>
<td>(1.0-3.0)</td>
<td>(0.0-0.5)</td>
<td>(3.0-3.0)</td>
<td>(1.0-3.0)</td>
<td>(2.2-12.6)</td>
<td>(0.0-0.0)</td>
<td>(2.2-13.6)</td>
</tr>
<tr>
<td>Alteplase</td>
<td>49.5</td>
<td>3.0</td>
<td>0.0</td>
<td>3.0</td>
<td>1.0*</td>
<td>3.5</td>
<td>0.5</td>
<td>3.9*</td>
</tr>
<tr>
<td>(n=16)</td>
<td>(43.0-66.0)</td>
<td>(0.0-3.0)</td>
<td>(0.0-1.0)</td>
<td>(3.0-3.0)</td>
<td>(0.0-3.0)</td>
<td>(1.1-9.6)</td>
<td>(0.0-2.1)</td>
<td>(1.2-15.2)</td>
</tr>
<tr>
<td>Combined</td>
<td>63.5</td>
<td>2.0</td>
<td>0.0</td>
<td>3.0</td>
<td>0.5†</td>
<td>0.7*</td>
<td>0.0†</td>
<td>0.6*</td>
</tr>
<tr>
<td>(n=14)</td>
<td>(59.0-74.0)</td>
<td>(1.0-3.0)</td>
<td>(0.0-0.5)</td>
<td>(3.0-3.0)</td>
<td>(0.0-2.0)</td>
<td>(0.0-9.8)</td>
<td>(0.0-0.0)</td>
<td>(0.0-9.7)</td>
</tr>
</tbody>
</table>

Data are median values with interquartile intervals in parentheses. CBF indicates cerebral blood flow.

*P<.05 vs control; †P<.01 vs control; ‡P<.05 vs alteplase group, all by Mann-Whitney test.
or does so poorly. Therefore, we measured selectively infarctions affecting cortex (supplied during "misery perfusion" by pial collaterals) and deep brain structures (supposedly perfused by end arteries only). Our results confirm the superior effect of EAA antagonism on the cortex (Table 2). It is of particular importance that only the combined treatment substantially reduced deep brain infarcts. We suggest two explanations for this: (1) Recanalization induced by thrombolysis allowed access of MK-801 to these areas soon enough to limit deterioration of the tissue caused by ischemia, while pial anastomoses are sufficient to deliver the compound to low-flow cortical areas. (2) The acidosis of ischemic areas is corrected quickly by reperfusion, thereby diminishing the intrinsic blockade of NMDA receptor (by acidosis) and replacing it immediately by extrinsic (MK-801–mediated) blockade so that glutamate excitotoxicity is eliminated during this particular period.

In our model, acidosis caused by hypoventilation during anesthesia with spontaneous respiration is constantly present and may attenuate the effect of NMDA antagonism. This, in combination with the fact that rat cortex has relatively poor collateral supply compared with gyrencephalic species, allows us to hypothesize that our model underestimates rather than overestimates the benefit of the combination of NMDA antagonism and thrombolysis compared with human embolic stroke. MK-801 was shown to induce degenerative changes in posterior cingulate cortex. In our opinion, an experiment on primates is needed to elucidate the risk/benefit ratio of noncompetitive NMDA blockade alone as well as the benefit of the combination of NMDA receptor blockade with thrombolysis. Zivin and Mazzarella recently reported improved clinical outcome and/or survival by the combination of dizocilpine and atepalase in a rabbit stroke model. Blockade of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor has also been shown to be effective in focal cerebral ischemia. Furthermore, augmentation of neuroprotection with NMDA receptor blockade by AMPA receptor antagonism on cultured neurons has been reported and may show superiority over single targeted EAA blockade.

Our results document comparable efficacy of delayed thrombolysis and EAA antagonism in this model and suggest that the combination of these two therapeutic approaches may yield additional benefit in the treatment of thromboembolic stroke, particularly in cases where deep brain (end-artery–supplied) structures are involved.

Acknowledgments

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References


**Editorial Comment**

While basic scientists work to increase our understanding of the mechanisms of ischemic injury and to develop and refine valid models of ischemic stroke, clinical scientists are just beginning to evaluate some of the many potential therapeutic agents for stroke. As yet there are no accepted treatments to treat acute ischemic stroke. Agents being tested at the present time can be placed in three categories: neuroprotective therapy, thrombolytic therapy, and anticoagulant therapy. Testing a single agent for stroke treatment has proven to be a difficult and challenging task. Reports from laboratory research are already exploring the combination of two or more therapeutic agents. Such research can be extremely helpful in designing future clinical studies. Useful information from these studies includes descriptions of the best time to administer each agent in relation to the other, drug interactions, and useful dosages. For instance, the neuroprotective agent dizocilpine was given after only 5 minutes of ischemia in the research reported here by Sereghy et al. The thrombolytic therapy, alteplase, was not given until 2 hours later. Could the dizocilpine be given any later and still have the same synergistic effect? Does early neuroprotective therapy increase the length of time that thrombolytic therapy can be effective? If an effective agent for stroke therapy ever is found, future clinical testing of agents may be made more difficult if control patients are receiving an active agent. This will increase the need for more data from the laboratory to help understand the relations between two or more therapeutic agents.

John R. Marler, MD, Guest Editor
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National Institute of Neurological Disorders and Stroke
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