Delayed Therapy of Experimental Ischemia
With Competitive N-Methyl-d-Aspartate
Antagonists in Rabbits

Kenneth P. Madden, MD, PhD; Wayne M. Clark, MD; Justin A. Zivin, MD, PhD

Background and Purpose: N-methyl-d-aspartate antagonists are effective in limiting ischemic damage to the brain and spinal cord if treatment is begun at time of ischemic injury. More clinically relevant delayed therapy has not been adequately investigated. We report the temporal profile of efficacy for two competitive N-methyl-d-aspartate antagonists in therapy of central nervous system ischemia.

Methods: CGS-19755 (30 mg/kg) or LY233053 (100 mg/kg) was administered 5, 30, or 60 minutes after reversible spinal cord ischemia in rabbits, induced by temporary occlusion of the infrarenal aorta. Duration of occlusion for individual animals was varied to provide a range of ischemia for each experimental group. The P50 represents the duration (in minutes) associated with a 50% probability of resultant permanent paraplegia. Neuroprotection is demonstrated if a drug prolongs the P50.

Results: CGS-19755 significantly prolonged the P50 (t test, P=.003) when given 5 minutes after ischemia, but not if delayed by 30 or 60 minutes (P50: control, 24.1; 5 minutes, 31.4; 30 minutes, 30.1; 60 minutes, 26.6). LY233053 was efficacious at 5 (P=.0008) and 30 (P=.002) minutes, but not at 60 minutes (P50: control, 26.8; 5 minutes, 39.4; 30 minutes, 36.0; 60 minutes, 25.6).

Conclusions: These competitive N-methyl-d-aspartate antagonists are effective in limiting ischemic damage, but protection is lost if therapy is not initiated within 60 minutes of injury. (Stroke 1993;24:1068-1071)

Key Words • neuronal damage • N-methyl-d-aspartate • spinal cord • rabbits

Excitatory amino acids appear to play a role in potentiation of neuronal ischemic damage. Excessive stimulation of excitatory neurotransmitter receptors is damaging to neurons, and this neurotoxicity can be blocked by specific antagonists. Similarly, antagonists of excitatory neurotransmitters have been shown to blunt cell injury in a variety of in vitro and in vivo models of neuronal ischemia.

The N-methyl-d-aspartate (NMDA) receptor, one of at least three subtypes of excitatory amino acid receptors, has been pharmacologically targeted in most prior studies of experimental ischemia. Both competitive and noncompetitive NMDA antagonists have been shown to display therapeutic efficacy in such models. It is not yet clear which type of drug may be more appropriately used in the clinical arena. This determination will likely be dependent on the spectrum of clinical side effects of individual drugs. Animal experiments suggest that competitive NMDA antagonists may be equally efficacious with less severe side effects, particularly with regard to sedation and production of a psychotomimetic state.

To date, therapeutic efficacy of excitatory amino acid antagonists in ischemic models has involved pretreatment of animals or very short delays between the onset of ischemia and drug administration. There are scant data, however, to support persistent benefit of these drugs after clinically relevant delays between ischemia and treatment.

In this study, we tested the delayed efficacy of two types of competitive NMDA antagonist, using a rabbit model of reversible central nervous system (CNS) ischemia. CGS19755 (cis-4-(phosphonomethyl) piperidine-2-carboxylic acid) and LY233053 (cis-(±)-4-(2H-tetraazol-5-yl)methyl piperidine-2-carboxylic acid) are recently developed competitive antagonists that effectively cross the blood-brain barrier after parenteral administration. We have previously reported dose-response relations for these two drugs when given 5 minutes after onset of ischemia, using this same animal model. Significant neuroprotection was demonstrated with 30-mg/kg doses of CGS-19755, but not with 10-mg/kg doses. Significant neuroprotection was demonstrated with LY233053 at 50 mg/kg. Even greater benefit was shown with 100 and 200 mg/kg, although these doses were essentially equivalent in efficacy. At the maximum doses tested, these drugs produced mild to moderate sedation and ataxia, with no significant effect on blood pressure or body temperature. This study examines the duration of effectiveness of these two drugs.

Materials and Methods

New Zealand White rabbits (2.5 to 3.0 kg) were anesthetized with halothane before invasive procedures. We made a 10-cm ventral midline incision to place a snare...
ligature about the infrarenal aorta. The snare device consisted of a large-bore Tygon tubing housing a loop of thin Tygon tubing that encircled the vessel. One end of the large tubing was exteriorized, allowing easy manipulation of the thin tubing within. Temporary spinal cord ischemia was accomplished by tightening the thin tubing for a predetermined duration, then releasing the snare to allow reperfusion. This ischemic duration was varied among the animals in each experimental group to provide a range of durations for the group. Eighteen hours after ischemia, the animals were examined for paraplegia. A binary scoring system was used, with animals rated (by blinded investigator) as either paraplegic or not paraplegic (includes animals with minimal motor function). Within each experimental group, the duration of ischemia producing a 50% probability of paraplegia in animals was termed the P50. We used an untreated group of animals as a control for each drug tested.

Three groups of animals were treated with an intravenous bolus of 30 mg/kg CGS-19755 (CIBA-GEIGY Corporation, Summit, NJ) at latencies of 5, 30, or 60 minutes after the onset of spinal cord ischemia. Three additional groups received 100 mg/kg LY233053 (Lilly Pharmaceuticals, Indianapolis, Ind) intravenously at either 5, 30, or 60 minutes after spinal cord ischemia. Efficacy of CGS-19755 or LY233053 was assessed by shift of the P50 in treated animals compared with the respective control groups. A rightward shift (greater P50) indicates that animals are able to tolerate longer durations of ischemia without resultant paraplegia.

Statistical significance of shifts in P50 were assessed by group t test and adjusted for multiple comparisons with the Bonferroni correction (P<.05). Computer-generated quantal dose-response curves, plotting “dose” of neurological insult (duration of spinal cord ischemia) versus functional outcome, were used to graphically display therapeutic efficacy. More detailed explanations of this data analysis method have been published.

Results

Table 1 lists duration of ischemia and functional outcome for four groups of animals. Eighteen untreated control rabbits received varying durations of spinal cord ischemia, with a resultant P50 (±SE) of 24.1±1.7 minutes. Ten rabbits received 30 mg/kg CGS-19755 5 minutes after initiation of ischemia, significantly prolonging the P50 to 31.4±1.5 minutes (P=.003). Ten rabbits received this drug 30 minutes after ischemia, with a P50 of 30.1±2.8 minutes. Ten animals received the drug 60 minutes after ischemia, with a P50 of 26.6±2.5 minutes. These prolongations did not reach statistical significance (power of test of each hypothesis, 0.96 and 0.093, respectively). Fig 1 graphically depicts the probability of resultant paraplegia after spinal cord ischemia for these groups.

Table 2 lists ischemic duration and functional outcome for four additional groups. Thirty untreated control rabbits received ischemia at varying durations, with a P50 of 26.8±1.9 minutes. In comparison, treatment with 100 mg/kg LY233053 in 15 rabbits 5 minutes after ischemia significantly prolonged the P50 to 39.4±2.7 minutes (P=.0008). Treatment with this dose 30 minutes after ischemia in 12 rabbits also resulted in significant prolongation of the P50 to 36.0±1.9 minutes (P=.002). Treatment 60 minutes after ischemia in 11 rabbits did not prolong the P50 (25.6±0.2 minutes; power of test of hypothesis, 0.037). Fig 2 graphically depicts the probability of resultant paraplegia after spinal cord ischemia for these groups.

Discussion

We tested the delayed efficacy of two types of competitive NMDA antagonist in these experiments. Both drugs were effective in limiting ischemic damage in this rabbit model, and each was most effective when given shortly after the ischemic insult. Both drugs generally maintained therapeutic efficacy after a 30-minute delay between ischemia and treatment. However, both drugs
lost this efficacy if treatment was delayed by 60 minutes. These data therefore reinforce the concept of a relatively short window of therapeutic effectiveness, which has been suggested in other models of CNS ischemia.9-11

Few investigations into delayed NMDA antagonist therapy of ischemia have been described. Most such studies have used pretreatment or therapy initiated within 5 minutes of ischemia. Roman et al12 reported neuroprotection using AP-7 given 15 minutes after cerebral ischemia. Grotta et al13 treated global ischemia in rats with CGS19755, measuring histological damage and learning ability. Treatment before ischemia resulted in histological protection, which was lost when therapy was delayed by 30 minutes. Delayed treatment did result in persistent benefit in learning ability, however. Most recently, Chen et al14 described loss of benefit after delayed therapy of CNS ischemia with D-CPPene, another competitive NMDA antagonist. Histological neuroprotection was noted when this drug was given before ischemia, but no significant protection could be demonstrated when therapy was delayed by 60 minutes. The present investigation also fails to demonstrate delayed efficacy of competitive NMDA antagonists. Only a single dose (presumably the most effective) of each drug was used in this study, however, and conclusions may not be applicable to other models or other species.

Competitive NMDA antagonists appear to be effective agents for use in clinical stroke. Their spectrum of side effects would appear to be acceptable in animal models, although clinical testing has barely begun. These data support their efficacy in treating CNS ischemia, and this effectiveness is maintained for at least short latencies between onset of ischemia and therapy.

![Graph showing relation of increasing probability of paraplegia with increasing duration of spinal cord ischemia for groups of control rabbits and those treated with CGS-19755 at delays of 5, 30, and 60 minutes. Each P50 (with standard error) is marked at the duration associated with a 50% probability of paraplegia. Treatment shifts the curve rightward, indicating greater tolerance of treated groups to ischemia. The degree of shift lessens with greater delays between ischemia and treatment.](image1)

![Graph showing relation of increasing probability of paraplegia with increasing duration of spinal cord ischemia, for groups of control rabbits and those treated with LY233053 at delays of 5, 30, and 60 minutes. Each P50 (with standard error) is marked at the duration associated with a 50% probability of paraplegia. Treatment at 5 and 30 minutes shifts the curve rightward, indicating greater tolerance of treated groups to ischemia. Treatment at 60 minutes does not shift the curve.](image2)

### Table 2. LY233053 (100 mg/kg): Duration of Ischemia and Functional Outcome After Three Treatment Delays

<table>
<thead>
<tr>
<th>Control</th>
<th>5 Minutes</th>
<th>30 Minutes</th>
<th>60 Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration (min)</td>
<td>Paraplegia</td>
<td>Duration (min)</td>
<td>Paraplegia</td>
</tr>
<tr>
<td>18</td>
<td>No</td>
<td>25</td>
<td>No</td>
</tr>
<tr>
<td>20</td>
<td>No</td>
<td>27</td>
<td>No</td>
</tr>
<tr>
<td>22</td>
<td>Yes</td>
<td>29</td>
<td>No</td>
</tr>
<tr>
<td>23</td>
<td>No</td>
<td>31</td>
<td>No</td>
</tr>
<tr>
<td>24</td>
<td>No</td>
<td>32</td>
<td>No</td>
</tr>
<tr>
<td>25</td>
<td>No</td>
<td>33</td>
<td>No</td>
</tr>
<tr>
<td>26</td>
<td>No</td>
<td>34</td>
<td>No</td>
</tr>
<tr>
<td>27</td>
<td>Yes</td>
<td>35</td>
<td>Yes</td>
</tr>
<tr>
<td>28</td>
<td>Yes</td>
<td>37</td>
<td>Yes</td>
</tr>
<tr>
<td>30</td>
<td>Yes</td>
<td>38</td>
<td>No</td>
</tr>
<tr>
<td>32</td>
<td>Yes</td>
<td>39</td>
<td>No</td>
</tr>
<tr>
<td>34</td>
<td>Yes</td>
<td>41</td>
<td>Yes</td>
</tr>
<tr>
<td>36</td>
<td>Yes</td>
<td>43</td>
<td>No</td>
</tr>
</tbody>
</table>
However, these data also support the emerging philosophy of stroke as a neurological emergency, requiring initiation of therapy on an urgent basis.

Acknowledgment
This study was supported by a grant provided by the Marshfield Medical Research Foundation (8551).

References

Editorial Comment

In the article by Madden et al, the utility of two N-methyl-D-aspartate (NMDA) receptor antagonists in limiting ischemic damage to rabbit spinal cord was tested. Both NMDA antagonists, LY223053 and CGS-19755, prolonged the P0 (time of aortic occlusion needed to result in 50% probability of permanent paraplegia). However, both drugs acted favorably only when given acutely, 5 to 30 minutes into the ischemic insult. The study adds to the plethora of pharmacological studies in diverse models of central nervous system ischemia in two ways: (1) demonstrating a role of the NMDA receptors also in spinal cord ischemic injury and (2) demonstrating beneficial effects in an ischemic injury not shown to possess the “penumbra phenomenon” so commonly discussed in brain ischemia. However, the article must be read with great caution for the following reasons. First, because of lack of a dose response, it is virtually impossible to know the efficacy of these drugs in this experimental paradigm. This is not an arbitrary issue, but an important one that has an impact on (1) therapeutic versus toxic effects; (2) the “therapeutic window”: higher doses may produce beneficial effects even at later time points; and (3) mechanism of action: many drugs, including NMDA receptor antagonists, possess different pharmacological actions at various dose ranges. Therefore, effects at high doses might present a secondary or tertiary feature of a chemical action of a drug completely unrelated to the main (claimed) feature. Second, the reader should also be cautioned to keep in mind that systemic drug administration may result in effects that are not necessarily at the site of organ injury; in this case, no proof has been provided that the drugs have gained access to the spinal cord and blocked local NMDA receptors at any time during the insult or thereafter. Although monitoring direct tissue levels of compound might be difficult (and sometimes impossible), simple methods can help; for example, extracting the drug from the tissue and subjecting the extract to an NMDA receptor binding assay (e.g., 3H-MK-801 displacement from spinal cord membranes) can be a useful adjunct to such studies. Furthermore, because drugs are provided during the insult (ischemia) period, one must always question whether the drug modifies the level of the insult itself. This issue commonly (if not too often) fails to reach the “Discussion” section, yet it may be the most pertinent explanation for the diminished deficit. For example, if neuronal firing in the spinal cord region subjected to ischemia is dramatically diminished via central effects of the drugs, a “quiescent effect” may bear on their durability to the ischemic period. Finally, in all pharmacological articles, seeking a perspective via-à-vis the many other drugs that have shown some modulatory effects on the same or similar model(s) will be useful, especially for the more naive reader who lacks such perspective.

Giora Feuerstein, MD, Guest Editor
SmithKline Beecham Pharmaceuticals
King of Prussia, PA

Reference
Delayed therapy of experimental ischemia with competitive N-methyl-D-aspartate antagonists in rabbits.

K P Madden, W M Clark and J A Zivin

Stroke. 1993;24:1068-1071
doi: 10.1161/01.STR.24.7.1068

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/24/7/1068

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/