Protective Effect of N-Methyl-D-Aspartate Antagonists After Focal Cerebral Ischemia in Rabbits

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We studied the efficacy of postischemic, systemic treatment with the N-methyl-D-aspartate (NMDA) receptor antagonists dextromethorphan and dextrorphan in a rabbit model of transient focal cerebral ischemia. Twenty-two rabbits underwent 1-hour occlusion of the left internal carotid and anterior cerebral arteries followed by 4.5 hours of reperfusion before sacrifice. One hour after the onset of ischemia, immediately after removing the arterial clips, the rabbits were blindly assigned to treatment with dextromethorphan (20 mg/kg i.v. loading dose followed by 10 mg/kg/hr maintenance infusion, n = 7), dextrorphan (15 mg/kg i.v. loading dose followed by 15 mg/kg/hr maintenance infusion, n = 7), or an equivalent volume of normal saline alone (n = 8). The maintenance infusion of drugs or saline was continued for the duration of the experiment. The formalin-fixed brains were analyzed with magnetic resonance imaging using coronal T2-weighted images, and ischemic neuronal damage was assessed on standard coronal hematoxylin- and- eosin-stained sections. The area of neocortical ischemic neuronal damage was significantly reduced in the groups treated with dextromethorphan (4.2%, p<0.01) and dextrorphan (6.1%, p<0.01) compared with the controls (36.2%). Magnetic resonance imaging demonstrated significantly smaller areas of cortical edema in the groups treated with dextromethorphan (14.6%, p<0.01) and dextrorphan (8.0%, p<0.01) compared with the controls (32.9%). These clinically tested antitussives with NMDA-antagonist properties may have therapeutic value in the treatment of human cerebrovascular disease. (Stroke 1989;20:1247-1252)

The excitatory amino acid glutamate has direct neurotoxic effects and may mediate hypoxic or ischemic neuronal damage. Recent evidence suggests that selective antagonism of the N-methyl-D-aspartate (NMDA) subclass of excitatory amino acid receptors can reduce such hypoxic-ischemic neuronal injury in culture, in brain slices, and in animal models. Since the competitive NMDA antagonists do not easily penetrate the blood–brain barrier, interest has focused on non-competitive NMDA antagonists such as ketamine, phencyclidine, and MK801. These lipophilic compounds can be administered systemically to act on the central nervous system. Dextromethorphan (p-3-methoxy-N-methylmorphinan, DM), a widely used antitussive, and its O-demethylated metabolite, dextrorphan (DX) are also noncompetitive NMDA antagonists. Using a rabbit model of transient focal cerebral ischemia, we have previously demonstrated that preischemic systemic treatment (and continued maintenance infusion) with DM and DX reduces cerebral injury. Obviously, a pharmacologic treatment that protects against cerebral damage when administered in a delayed fashion following the ischemic insult would have greater therapeutic potential for clinical stroke. Therefore, we examined the effect of postischemic treatment using DM and DX in our rabbit model. We have reported some of these results in abstract form.

Materials and Methods

Our methods have been described. Twenty-two male rabbits weighing 2.5–3.5 kg were anesthetized with 3% halothane delivered by mask. After
trachaeostomy, the rabbits were paralyzed with pancuronium bromide and artificially ventilated. Anesthesia and paralysis were maintained for the duration of the experiment using 0.5% halothane in 100% oxygen and supplemental intravenous pancuronium bromide as needed. Mean arterial blood pressure (MABP), rectal body temperature, and end-expired CO2 were regulated as noted previously. The rabbits underwent 1-hour transient occlusion of the left internal carotid and left anterior cerebral arteries. Via a microsurgical, retroorbital approach, the rabbits underwent 1-hour transient occlusion of the left internal carotid and left anterior cerebral arteries.

The rabbits were randomized into DM (n=7), DX (n=7), or normal saline (control, n=8) groups. One hour after the onset of arterial occlusion, immediately after removing the clips, rabbits were treated with a 20 mg/kg i.v. loading dose followed by a 10 mg/kg/hr i.v. maintenance infusion of 0.4% DM-HBr, a 15 mg/kg i.v. loading dose followed by a 15 mg/kg/hr i.v. maintenance infusion 0.4% DX-HCl, or an equivalent volume of normal saline according to group assignment. The loading dose was given over 0.5 hour, and the maintenance infusion continued for the duration of the experiment. Four and one half hours after reperfusion, the rabbits were killed with sodium pentobarbital and perfused with saline followed by 10% buffered formalin; the brains were removed immediately to be stored in formalin.

Each rabbit brain was studied with magnetic resonance imaging (MRI), obtaining T2-weighted images of 5-mm-thick slices, using a repetition time of 2,500 msec and an echo time of 100 msec. The areas of high-intensity signal (representing edema) were delineated on the two most anterior coronal levels by projecting the MRI with a standard darkroom enlarger onto a transparency. Using a magnetic digitizing tablet, the high-intensity areas were expressed as a percentage of the total area of neocortex for each individual coronal level and for both levels together. After MRI, the brains were prepared for histopathology as described. Two standard levels were chosen for histologic examination: Level 1, 3 mm anterior to the anterior commissure and Level 2, at the anterior commissure. Ischemic neuronal damage (IND) characterized by moderate to severe neuronal shrinkage, increased nuclear basophilia, and nuclear pyknosis, was assessed in the left neocortex and striatum. These microscopic observations were delineated on an enlarged transparency of the slide. Using a magnetic digitizing tablet, the area of IND was expressed as a percentage of the total area of the left neocortex or striatum at each individual coronal level and for both levels together.

The data are presented as mean±SEM. IND and MRI results were statistically evaluated as independent measures of cerebral ischemia using analysis of variance and Fisher’s least significant difference tests for multiple comparisons; p<0.05 was considered to be significant.

### Results

Systemic parameters, including heart rate, MABP, body temperature, end-expired CO2, arterial pH, Pao2, Paco2, and hematocrit were not significantly different among groups before occlusion, during occlusion, or during reperfusion. All groups received equivalent volumes of fluid over the course of the experiment. Administration of DM or DX did not affect systemic parameters including heart rate, MABP, body temperature, end-expired CO2, or arterial blood gases (Table 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>At occlusion</th>
<th>After loading dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>DM</td>
<td>DX</td>
</tr>
<tr>
<td>246±18</td>
<td>241±11</td>
<td>235±17</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>63±2</td>
<td>61±3</td>
</tr>
<tr>
<td>Temperature (° C)</td>
<td>37.6±0.3</td>
<td>37.6±0.01</td>
</tr>
<tr>
<td>End-expired CO2 (mm Hg)</td>
<td>36±1</td>
<td>37±2</td>
</tr>
<tr>
<td>pH</td>
<td>7.37±0.03</td>
<td>7.39±0.03</td>
</tr>
<tr>
<td>Pao2 (mm Hg)</td>
<td>369±33</td>
<td>391±41</td>
</tr>
</tbody>
</table>

Values are mean±SEM. DM, dextromethorphan (n=7); DX, dextrorphan (n=7); control, normal saline (n=8); MABP, mean arterial blood pressure.
and DX 61.0% [not significant] compared with control 67.6%) and for the two levels together (DM 36.7% [p<0.05] and DX 48.6% [not significant] compared with control 72.6%) (Figure 1). There was no significant difference between DM and DX in the degree of cerebral protection against striatal ischemia.

High-intensity MRI lesions were found predominantly in the cortex, with only occasional involvement of the striatum. These lesions were localized to the anterior portion of the middle cerebral artery distribution ipsilateral to the arterial occlusion. As shown in Figures 2 and 3, MRI demonstrated significantly smaller areas of cortical edema in the DM (14.6%, p<0.01) and DX groups (8.0%, p<0.01) than in the control group (32.9%). The benefit of DM and DX was present at both Level 1 (DM 19.3% [p<0.05] and DX 10.9% [p<0.01] compared with control 36.2%) and at Level 2 (DM 11.5% [not significant] and DX 6.4% [p<0.05] compared with control 28.8%) (Figure 3). There was no significant difference between DM and DX in the degree of cerebral protection against ischemic edema on MRI.

Discussion

DM and its O-demethylated conjugate, DX, are dextrorotatory morphinan compounds devoid of the troublesome narcotic side effects of their levorotatory analogues. DM and DX have been clinically tested and are relatively safe in conventional antitussive doses. DM and DX have been shown to attenuate NMDA-induced excitation using electrophysiological techniques in rat spinal cord, in mouse neuronal culture, and in rat and guinea pig brain slices. DM and DX have anticonvulsant properties and have been shown to reduce seizures in several rodent epilepsy models. Recent evidence suggests that these compounds are noncompetitive NMDA antagonists and may block the NMDA receptor-channel complex by binding to the phencyclidine site.

FIGURE 1. Bar graph of mean±SEM percent area with ischemic neuronal damage on histologic examination at each coronal level in rabbits. CX, cortex; STR, striatum. Dextromethorphan (filled bars, n=7) and dextrorphan (shaded bars, n=7) reduce ischemic neuronal damage compared with saline-treated control group (open bars, n=8). *p<0.05 different from control by analysis of variance and Fisher's least significant difference test.

FIGURE 2. Magnetic resonance images of rabbit brains in coronal plane. T2-weighted images; repetition time 2,500 msec; echo time 100 msec; 5-mm-thick slices. Dextromethorphan- (a) and dextrorphan- (b) treated rabbits show little ischemic edema (arrows) compared with normal saline-treated controls (c).

Our present study demonstrates that posts ischemic treatment with systemic DM and DX protects against the development of IND and ischemic edema evaluated 5.5 hours after the onset of transient focal cerebral ischemia. The degree of necocortical histopathologic protection provided by DM (88%) and DX (83%) is comparable to that found using preischemic treatment with DM (76%) and DX (79%). While we have not delineated the precise mechanism of cerebral protection for DM or DX, our present results are consistent with the notion that NMDA antagonists ameliorate hypoxic-ischemic neuronal injury. Choi and his group have demonstrated that DM and DX protect neurons in culture against NMDA-mediated toxicity, glutamate-induced injury, and hypoxic damage. DM and DX have been shown to protect against cerebral injury following transient focal ischemia in rabbits and in rats. Other NMDA antagonists have also been demonstrated to attenuate hypoxic-ischemic injury in culture, in brain slices, and in...
animal models.\textsuperscript{1,2,10,11} Furthermore, delayed treatment with systemically administered MK-801 (a noncompetitive NMDA antagonist) and the competitive NMDA antagonists CGS 19755 and 4-(3-phosphonopropyl)-2-piperazine-carboxylic acid (CPP) also have been reported to decrease ischemic damage in several animal models of focal and global cerebral ischemia.\textsuperscript{2,6,8,9,32-34} However, the evidence that NMDA antagonists reduce ischemic brain injury is still controversial. Other studies have failed to show a benefit of both competitive and noncompetitive NMDA antagonists (including MK801) against hypoxic-ischemic brain damage in hippocampal slices or in animal models.\textsuperscript{35-39}

The mechanism of postischemic cerebral protection using DM or DX is not known. The drugs may antagonize a glutamate- or NMDA-receptor-mediated increase in calcium permeability, thereby preventing delayed neuronal death.\textsuperscript{5,40,41} We recognize that the protective effect of DM and DX may not be related to their NMDA-antagonist properties. Another possibility is that DM and DX improve postischemic hypoperfusion by increasing collateral blood flow or by decreasing neuronal metabolic requirements. This seems less plausible in view of a recent report suggesting that the NMDA antagonist thienylcyclohexylpiperidine attenuates focal cerebral ischemia in the rat brain without causing general metabolic depression or direct cerebrovascular action.\textsuperscript{42} Since DM and DX also have antiepileptic properties,\textsuperscript{26,28-30} it is conceivable that the drugs prevent seizure-induced neuronal injury. We believe that this explanation is unlikely in view of the absence of clinical seizures or electroencephalographic epileptiform activity in our rabbit model.\textsuperscript{16,18}

While a preliminary report of one study suggests that the protective effect of MK801 is mediated by hypothermia,\textsuperscript{39} we found no significant differences in rectal body temperature between drug- and saline-treated groups at any time during the experiment. It is possible that rectal temperatures do not reflect brain temperatures, and in our future studies we will attempt to measure temporalis muscle temperature as a better indicator of brain temperature. The protective effect of systemic DM in our present study may be mediated via DX after O-demethylation in the liver.\textsuperscript{43-45} However, measurements of plasma and brain levels of DM and DX suggest that DM has an independent cerebral protective effect (unpublished observations).

The lesser degree of protection against IND in the striatum after postischemic treatment with DM and DX is consistent with our preischemic treatment studies using DM and DX.\textsuperscript{16-18} Both preischemic and postischemic treatment experiments using MK-801 have also demonstrated a relative lack of protection in the ischemic striatal territory.\textsuperscript{7-9} Since the striatum has a lower concentration of NMDA receptors than the cortex,\textsuperscript{46,47} this lesser degree of protection in the striatum may reflect the importance of other non-NMDA mechanisms in causing ischemic neuronal injury. Alternatively, collateral blood flow in the striatum may be insufficient to allow protection by systemically administered NMDA antagonists.

Previous studies have shown that MRI is a sensitive measure of early cerebral ischemia, with the high-intensity signal on T2-weighted images representing increased water content in the brain.\textsuperscript{48} Previous work in our laboratory has demonstrated that in vivo MRI images of ischemic rabbit brains have a high correlation with MRI images performed after fixation.\textsuperscript{20} We also showed in a previous series of anesthetized rabbits that the combination of arterial occlusion in our model results in MRI high-intensity lesions in the anterior portion of the middle cerebral artery distribution, ipsilateral to the arterial occlusion. Rabbits that underwent an identical surgical procedure, including dissection of the vessels, but without arterial occlusion showed no significant MRI lesion. Furthermore, we demonstrated that preischemic treatment with DM and DX reduces the area of cortical edema on MRI.\textsuperscript{16,17} Our present study shows that postischemic treatment with DM and DX 1 hour after the onset of arterial occlusion can also attenuate the degree of ischemic edema on MRI. It is possible that NMDA receptor blockade prevents ischemia-induced alterations in neuronal or glial membrane permeability and secondary fluid shifts, but other non-NMDA mechanisms may underlie this protective effect against ischemic edema.

There are several limitations to our present study. While we have demonstrated protection against IND and ischemic edema at 5.5 hours after the ischemic insult, it is unknown whether this protective effect on histopathology and MRI will hold up at 1 day or 1 week after ischemia. Another issue is whether DM and DX can improve clinical neurologic deficits following focal ischemia, and we are
currently conducting a study in which anesthesia is discontinued following transient ischemia to assess the effect of these drugs on neurologic outcome in our rabbit model. Since our present study was conducted under anesthesia with halothane, an agent known to have adverse effects on cerebral ischemia, it is possible that DM or DX specifically protect against halothane’s negative effects and they may not prove to be efficacious under other conditions. Finally, it is important to determine how long after ischemia treatment with DM or DX can be delayed and still protect against ischemic cerebral injury.

Calcium channel blockers, prostacyclin, barbiturates, inhibitors of phospholipase, and other pharmacologic agents have been shown to improve neurologic outcome in animal models when administered before or, in some cases, after the ischemic event. However, clinical trials with these same drugs have been disappointing. It remains to be determined whether DM and DX will prove beneficial in humans with cerebral ischemia and whether these drugs will be tolerated in clinical trials at doses sufficient to provide neuronal protection.

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References


**Key Words**: animal models • cerebral ischemia • rabbits • NMDA
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