Dextromethorphan Protects Against Cerebral Injury Following Transient Focal Ischemia in Rabbits

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We investigated dextromethorphan, both a dextrorotatory opioid derivative and a clinically tested N-methyl-d-aspartate (NMDA) receptor antagonist, in a rabbit model of transient focal cerebral ischemia. Fourteen rabbits were randomly assigned to treatment with a 20 mg/kg i.v. loading dose followed by a 10 mg/kg/hr infusion of 0.4% dextromethorphan in normal saline or with an equivalent volume of normal saline alone. One hour after treatment, the rabbits underwent a 1-hour occlusion of the left internal carotid and anterior cerebral arteries followed by 4 hours of reperfusion. The seven dextromethorphan-treated rabbits showed a significant decrease in the area of neocortical severe ischemic neuronal damage (10.5%) compared with the seven normal saline-treated controls (49.6%, p<0.001). The dextromethorphan-treated rabbits also demonstrated significantly smaller areas of cortical edema (10.2%) on magnetic resonance imaging than the controls (38.6%, p<0.01). Analysis of somatosensory evoked potentials revealed recovery of the ipsilateral amplitude to contralateral values within 5 minutes of reperfusion in the dextromethorphan-treated rabbits but not in the controls (p<0.01). In our rabbit model of transient focal cerebral ischemia, dextromethorphan appears to protect the brain against ischemic neuronal damage and edema, as well as to promote neurophysiologic recovery. This clinically available drug should be further investigated as having potential therapeutic value in the treatment of stroke. (Stroke 1988;19:1112-1118)

Cerebrovascular disease is the third leading cause of death in the United States and is a major cause of disability. Thromboembolism is responsible for three fourths of all cerebrovascular disease, with intracranial hemorrhage and subarachnoid hemorrhage comprising most of the remainder. During intracranial vascular surgery on aneurysms and arteriovenous malformations, it is often necessary to temporarily or permanently occlude important arteries. While deliberate transient or even permanent arterial clipping is frequently tolerated, there is a significant risk of serious ischemic complications with major neurologic sequelae. At the present time, there are very few treatment modalities available that are of proven efficacy in ameliorating the devastating effects of cerebrovascular disease. Glutamate, an excitatory amino acid neurotransmitter, has been shown to have neurotoxic effects and may mediate the neuronal injury that follows ischemia or hypoxia. Recent studies have demonstrated that selective antagonism of the N-methyl-d-aspartate (NMDA) subclass of excitatory amino acid receptors can attenuate glutamate-induced neurotoxicity, ischemic neuronal damage, or hypoxic brain injury. Of particular interest are the noncompetitive NMDA antagonists such as ketamine, phencyclidine (PCP), and MK-801 since these lipophilic compounds readily penetrate the central nervous system and can be administered systemically. Dextromethorphan (d-3-methoxy-N-methylmorphinan, DM), a widely used antitussive, and its O-demethylated conjugate, dextrorphan (DX), are also noncompetitive NMDA antagonists, and they most likely block the NMDA receptor-channel complex by binding to the PCP site. Both DM and DX have been shown to protect neurons in culture against hypoxic injury. Our study demonstrates the protective effect of DM in a rabbit model of transient focal cerebral
ischemia. We have reported some of these results in preliminary form.\textsuperscript{16,17}

**Materials and Methods**

Fourteen male New Zealand white rabbits (2–3 kg) were sedated with 2 mg diazepam and anesthetized with 3% halothane delivered by mask. A catheter was inserted into the ear vein. The rabbits underwent tracheostomy, paralysis with 0.2 mg/kg i.v. pancuronium bromide, and artificial ventilation using 0.5% halothane in 100% oxygen. A cut-down catheter was placed in the femoral artery. Body temperature was measured with a rectal probe and kept between 38.5° and 39.5° C with a warming pad. A capnograph was used to continuously monitor end-expired CO\textsubscript{2}, and this was kept between 35 and 40 mm Hg by adjusting the respiratory rate. Arterial blood gases were measured before surgery and 2 hours after arterial occlusion. Base deficit was corrected when necessary with intravenous sodium bicarbonate. Electrocardiogram and mean arterial blood pressure (MABP) were monitored continuously, and MABP was kept at >60 mm Hg using intravenous normal saline (NS) as needed.

Rabbits were randomized into experimental (DM) or control (NS) treatment groups. The rabbits were blindly given either a 20 mg/kg i.v. loading dose of 0.4% DM in NS over 20 minutes followed by a 10 mg/kg/hr infusion of 0.4% DM in NS or an equivalent volume of NS alone. In a separate series of unanesthetized rabbits, we found this DM dose to be near the maximum tolerated without the development of toxic side effects. At a bolus DM dose of >20 mg/kg i.v. or an infusion of >10 mg/kg/hr, we noted the rabbit first developed ataxia; with increasing doses we observed muscular tremor, catatonia, focal motor seizures, hypotension, opisthotonos, and apnea.

One hour after initiation of the treatment, the rabbits underwent 1 hour of transient transorbital occlusion of the left internal carotid artery and the distal A1 segment of the anterior cerebral artery using Yasargil miniature aneurysm clips.\textsuperscript{18} In a pilot study, we occluded various cerebral arteries in anesthetized rabbits and determined that this combination of arterial occlusion resulted in a reproducible area of ischemic damage in the area supplied by the anterior portion of the middle cerebral artery and the lenticulostrate vessels.

The rabbit’s head was immobilized in a stereotactic frame, and the scalp was incised to expose the underlying skull. Stainless steel screw electrodes were placed bilaterally over the frontal and parietal areas for recording somatosensory evoked potentials (SEPs). Stimulating electrodes were placed over the median nerve of each forepaw. Square-wave stimuli of 0.25 msec duration and 10 mA were delivered at 2.1/sec to each median nerve while SEPs were recorded from the contralateral frontal region with a Nicolet Pathfinder II electrodiagnostic system (Madison, Wisconsin) using the following parameters: band-pass filter 30–1500 Hz, recording duration 20 msec, and sensitivity 50–500 mV. Two hundred fifty responses were averaged. SEPs were recorded before administration of DM or NS, after administration of DM or NS, before arterial occlusion, 10 minutes after occlusion, 50 minutes after occlusion, and then after 5 minutes, 1, 2, 3, and 4 hours of reperfusion following removal of the aneurysm clips.

For analysis of SEP, the amplitude of the primary cortical potential was measured from the peak of the major positive deflection to the trough of the major negative deflection.\textsuperscript{19} SEP amplitudes were then expressed as a percentage of the initial (pre-treatment) amplitude for purposes of statistical analysis. Latencies of the major positive and major negative deflections were determined. In one NS-treated rabbit SEPs were not recorded throughout the experiment, and this rabbit was not included in the SEP results.

Four hours after reperfusion, the rabbits were killed with 25 mg/kg sodium pentobarbital. Perfusion was performed through a left ventriculostomy using 200 ml NS followed by 300 ml 10% phosphate-buffered formalin. Brains were removed immediately after perfusion and stored in 10% buffered formalin.

After storage in 10% formalin for 3–8 (mean 5) days, each rabbit brain was studied using magnetic resonance imaging (MRI). MRI studies of 18 rabbits with similar ischemic lesions in our laboratory have shown no significant differences in relative signal intensity or area between premortem and postfixation images.\textsuperscript{20} We used a 1.5-T General Electric Signa system (Milwaukee, Wisconsin) with a 6-in.-diam. saddle coil (positioned at magnetic isocenter), a 16-cm field of view, a 256x256 matrix (0.625 mm pixel diameter), and 2 excitations/projection (17 minute, 10 second scan time). Spin-echo, T2-weighted images, using a repetition time (TR) of 2000 msec and an echo time (TE) of 80 msec, were obtained in both the horizontal and coronal planes. Using 5-mm-thick contiguous slices, the cerebral hemispheres were visualized on four coronal and three horizontal images. The three most anterior coronal levels and the two most inferior horizontal levels were evaluated without knowledge of the treatment group. The areas of high-intensity signal (representing edema) were mapped by projecting the MRIs with a standard darkroom enlarger onto a transparency. Using a digitizer and an IBM PC XT computer, the areas of high-intensity signal were expressed as a percentage of the total area of the neocortex. Coronal MRIs were not performed on one DM rabbit due to technical problems.

After MRI, the brains were blocked in the coronal plane, embedded in paraffin, sectioned at 6-\textmu m thicknesses, and stained with hematoxylin and eosin. Four coronal levels were chosen for histologic examination: Level 1, 3 mm anterior to the anterior commissure (AC); Level 2, at the AC; Level 3, 3
mm posterior to the AC; and Level 4, 6 mm posterior to the AC. Ischemic neuronal changes were assessed in a blinded fashion (without knowledge of treatment group or MRI or SEP findings) in the left neocortex, caudate, and putamen at each level. Severe ischemic neuronal damage (SIND) was considered to be Grades 2 and 3 neuronal ischemic changes according to a previously described grading system. These observations were made from microscopic slices and were delineated on a photographic print of the slide. Using a digitizer and an IBM PC XT computer, the area of SIND was expressed as percentage of the total area of the left neocortex, caudate, or putamen.

SEP, MRI, and SIND results were statistically evaluated as independent measures of cerebral ischemia using two-tailed t tests and Mann-Whitney U tests.

**Results**

There were no significant differences in heart rate, MABP, end-expired CO₂, pH, or temperature between DM and NS rabbits. However, there did appear to be a trend toward a decrease in MABP with DM treatment.

On histopathologic examination there was a significant decrease in the neocortical area showing SIND in the DM group compared with the NS group at each coronal level examined (Figure 1). The average area of neocortical SIND for coronal Levels 1–4 combined was 10.5% in the DM group (range 1.6–19.6%) and 49.6% in the NS group (range 4.2–78.5%, p<0.001; Figure 2). In the striatum, the DM group had a smaller area of SIND averaged over all coronal levels and at each coronal level than the NS group, but this difference reached significance only at the most posterior level for both the caudate and the putamen (Figures 1 and 2).

MRI high-intensity lesions were predominantly localized to the cortex, with infrequent involvement of the caudate or the putamen. These lesions occurred in the anterior portion of the middle cerebral artery distribution ipsilateral to the arterial occlusion. On coronal images the DM group had significantly smaller areas of cortical high-intensity lesions (10.2%) than the NS group (38.6%, p<0.01; Figures 3 and 4). The horizontal images also demonstrated significantly smaller areas of high-intensity lesions in the DM group (23.1%) than in the NS group (50.5%, p<0.05; Figures 3 and 4).

Typical examples of the left hemisphere SEPs for single DM and NS rabbits are shown in Figure 5. SEP amplitudes before treatment with DM or NS were not significantly different between groups or between cortexes. Figure 6 shows the change in SEP amplitudes expressed as a percentage of the initial value for each group over the course of the experiment. After intravenous infusion of DM, there was an increase in the amplitude of the SEP bilat-
Figure 3. Magnetic resonance images of rabbit brains. T2-weighted images, (repetition time 2000 msec, echo time 80 msec) 5-mm-thick slices in coronal (a, b) and horizontal (c, d) planes. Dextromethorphan-treated rabbits (a, c) demonstrated little ischemic edema (arrows) compared with normal saline-treated controls (b, d).

Figure 4. Mean + SEM percent area with cortical edema over four coronal and three horizontal magnetic resonance images. Seven rabbits in normal saline-treated (shaded bars) (coronal, horizontal) and dextromethorphan-treated (DM) (shaded bars) (horizontal) groups; six rabbits in DM (coronal) group. DM protected against ischemic edema in both coronal (p<0.01) and horizontal (p<0.05) planes; two-tailed t test.

Discussion

Using our rabbit model of transient focal cerebral ischemia, our results show that intravenous DM administered before, during, and after arterial occlusion can significantly reduce neocortical ischemic neuronal damage, can attenuate ischemic cerebral edema seen on MRI, and can enhance neurophysiologic recovery of the ipsilateral SEP. Other preliminary evidence using an in vivo rat model of hypoxic-ischemic injury suggests that DM may reduce cerebral damage. \(^{22}\) Both DM and DX also protect against hypoxic injury in neocortical tissue culture. \(^{8}\)

The mechanism of brain protection in our rabbit model is not known with certainty. However, recent work suggests that DM and DX act on the NMDA receptor-channel complex by binding noncompetitively to the PCP site. \(^{12,15}\) DM and DX both reduce NMDA-induced excitation in the spinal cord, \(^{22}\) and DX attenuates the depolarization and increased conductance caused by NMDA in neocortical cell culture and cortical slices. \(^{7,24}\) Other specific NMDA antagonists have also been demonstrated to reduce glutamate-induced neurotoxicity, ischemic neuronal injury, and hypoxic cerebral damage, both in vitro and in vivo using animal models. \(^{5,8-10,25,26}\)

Alternatively, DM might have ameliorated cerebral ischemia in our experiment by some other mechanism, such as increasing collateral cerebral blood flow or reducing cerebral metabolic requirements. It is less likely that DM prevented seizure-induced neuronal injury by acting as an anticonvulsant \(^{27}\) since we have never seen clinical seizures or electroencephalographic (EEG) epileptiform activity in our rabbit model (unpublished observations).

Our study confirms the usefulness of our rabbit model of transient focal cerebral ischemia in evaluating potential therapeutic modalities. With seven rabbits in a group, we demonstrated a clear beneficial effect of DM on histopathology, MRI lesions, and electrophysiologic function. Other reports suggest that severe ischemic neuronal damage (Grades 2 and 3), even as early as 5 hours after the onset of
ischemia, may represent irreversible neuronal injury. The relative lack of protection by DM against neuronal damage in the striatum compared with the neocortex has also been demonstrated for MK-801 in a cat model of focal cerebral ischemia. This effect may relate to a lower density of NMDA receptors. Striatal NMDA binding in rat brains is only 60% that of Layers 1–3 in the cortex. High-affinity [3H]DM sites are more concentrated in the guinea pig neocortex than in the caudate and putamen. Furthermore, autoradiographic studies revealed dense labeling of tritiated PCP in the cerebral cortex, with only moderate labeling in the striatum. It is also possible that NMDA receptors have a less important role in mediating striatal ischemic neuronal damage. In fact, there is some evidence that increased dopaminergic activity can uncouple posts ischemic metabolism and blood flow in the striatum, resulting in selective ischemic injury. Finally, the lesser degree of cerebral protection in the striatum compared with the neocortex may be secondary to more severe ischemia in our model due to poorer collateral blood supply that cannot be reversed by NMDA antagonism.

MRI is a sensitive means of evaluating early cerebral ischemia. We have previously shown that in vivo MRIs of ischemic rabbit brains correlate highly with MRIs obtained after fixation. The high-intensity signal on T2-weighted MRI likely represents an increase in the water content of the ischemic brain due primarily to glial swelling and interstitial edema. We have found high-intensity signals on MRI to correspond closely to neuropil vacuolization but less consistently to neuronal injury in a recent study of histologic changes in rabbit cerebral ischemia. The lack of MRI abnormalities in the basal ganglia of our control rabbits despite extensive ischemic neuronal injury is noteworthy in

**FIGURE 5.** Examples of left hemisphere somatosensory evoked potentials (SEPs) in one dextromethorphan-treated (DM) and one normal saline-treated (NS) control rabbit. DM increased amplitude of SEP 1 hour after treatment. DM did not protect against loss of SEP after arterial occlusion but promoted recovery of SEP with reperfusion. In control, SEP disappeared after arterial occlusion and recovered to only one-sixth preocclusion value. P, major positive cortical deflection; N, major negative cortical deflection.

**FIGURE 6.** Mean normalized somatosensory evoked potential (SEP) amplitude as percentage of initial SEP in ipsilateral left (filled symbols) and contralateral right hemisphere (open symbols) over time course of experiment in hours. Seven rabbits in dextromethorphan-treated (circles) (DM) and six rabbits in normal saline-treated (triangles) (NS) group. DM enhanced bilateral SEP before arterial occlusion. After occlusion, left SEP disappeared in both DM and NS groups. Within 5 minutes of reperfusion left SEP in DM group recovered to contralateral values, while in NS group left cortical SEP recovered to <50% of contralateral values after 2 hours of reperfusion (p<0.05, Mann-Whitney U test).
this regard. It is possible that these areas have less edema because of differences in blood flow during reperfusion, or that small changes in the water content of the striatum are below the resolution of our scanners. We also cannot rule out early cerebral infarction occurring in these areas of MRI high-intensity signal since our histologic methods at 5 hours postmortem cannot distinguish reversibly damaged from infarcted tissue.

SEPs have been shown to be a reliable measure of focal cerebral ischemia. While the location of histologic ischemic neuronal damage or MRI evidence of cerebral edema in our rabbits appears to be anterior to the somatosensory cortical region, the change in SEP amplitude following arterial occlusion may be caused by ischemia in the white matter afferent pathways, such as the internal capsule. This might cause cortical deafferentation rather than direct cortical ischemia in the somatosensory area. We found that intravenous DM administered before arterial occlusion caused a bilateral increase in the amplitude of the SEP. PCP, ketamine, and MK-801 have all been shown to increase the amplitude of certain-frequency EEG activity. The potentiation of the SEP amplitude may relate to antagonism of the NMDA receptor-channel complex and disinhibition of somatosensory pathways. DM did not protect against loss of the SEP after arterial occlusion; however, it allowed the SEP amplitude to return to contralateral values more quickly after reperfusion. While it is possible that the rapid recovery of the SEP in the ischemic brains of DM-treated rabbits relates to delivery of DM during reperfusion and nonspecific potentiation of electrical activity in the remaining viable neurons, our histologic and MRI findings favor an alternative hypothesis, that DM may have protected ischemic neurons during the period of temporary occlusion, allowing neurophysiologic recovery after reperfusion.

Further studies using DM and its derivative DX are presently being done to confirm these drugs’ protective effects under different conditions of focal ischemia and to elucidate their mechanism of action in vivo. Preliminary results of these experiments suggest that both DM and DX significantly reduce neocortical severe ischemic neuronal damage when administered in a delayed fashion 1 hour after the ischemic insult, after reperfusion has begun. We believe these clinically available compounds may have potential therapeutic value in human cerebral ischemia; clinical trials should be considered.

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