Reduction of ischemic spinal cord injury by dextrorphan: Comparison of several methods of administration

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Objectives: We investigated the effect of dextrorphan, an N-methyl-D-aspartate receptor antagonist, on the reduction of ischemic spinal cord injury and the safe clamping time after various methods of administration.

Methods: Spinal cord ischemia was induced in New Zealand White rabbits by infrarenal aortic clamping and animals were divided into 5 groups. Group A (n = 15) received simple clamping. Groups B (n = 20) and C (n = 35) received dextrorphan pretreatment (10 mg/kg), followed by continuous intravenous or intra-aortic infusion (1 mg/min), respectively. Group D (n = 25) received the same dextrorphan pretreatment and bolus intra-aortic injection at clamping (1 mg per minute of clamping time). Group E (n = 15) received bolus intrathecal injection of dextrorphan (0.2 mg/kg). Each dextrorphan-treated group had a small group of control animals (n = 5). The neurologic status was assessed by the Johnson score (5 = normal, 0 = paraplegic) 48 hours after unclamping, and animals were put to death for histopathologic examination.

Results: All dextrorphan-treated groups showed better neurologic function than the respective control animals (P < .001 vs groups B, C, and D; P = .014 vs group E). The order of efficacy of dextrorphan (as revealed by the average of neurologic status) was as follows: group C > group D (P = .017, after 50 minutes of clamping), group D > group B (P = .014, after 45 minutes of clamping), and group B > group E (P < .001, after 40 minutes of clamping). Histopathologic findings did not necessarily correspond with hind-limb neurologic function.

Conclusions: Dextrorphan reduced the physical findings associated with ischemic spinal cord injury, and continuous intra-aortic infusion prolonged the safe clamping time significantly more than delivery by other routes.

Spinal cord ischemia is a severe complication of surgery on the descending aorta and thoracoabdominal aorta that has a major effect on the postoperative quality of life.1,2 Once the spinal cord becomes ischemic, the excitatory neurotransmitter glutamate is released from neuronal synapses and activates intracellular Ca2+ influx. This Ca2+ influx is thought to trigger a chain of reactions that lead to neuronal death.3 Protection of the central nervous system with noncompetitive antagonists of N-methyl-d-aspartate (NMDA), a glutamate receptor subtype, has been documented.4,5 Many drugs, including, dextromethorphan,6 dextrorphan,7-9 memantine,10 and riluzole,11 are known as noncompetitive NMDA receptor antagonists and have been shown to reduce paraplegia after spinal cord ischemia. Dextrorphan has been shown to protect the spinal cord against 158 to 259 minutes of ischemia. However, no study
demonstrating the maximum length of the safe clamping time achievable by dextrorphan has been reported. We examined the effect of dextrorphan on spinal cord ischemic injury and sought to prolong the safe aortic clamping time by testing various drug delivery methods, such as intravenous infusion, intra-aortic injection, and intrathecal injection.

Materials and Methods

Animal Care and Surgical Procedure

We used male New Zealand White rabbits weighing 2.5 to 3.5 kg. All animals received humane care in compliance with the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996, and the “Guidelines for Animal Experimentation” of Hamamatsu University School of Medicine.

Animals were randomly divided into 5 groups. Group A (n = 15) received simple infrarenal aortic clamping, group B (n = 20) received continuous intravenous infusion of dextrorphan, group C (n = 35) received continuous intra-aortic infusion of dextrorphan, group D (n = 25) received a bolus intra-aortic injection of dextrorphan, and group E (n = 15) received a bolus intrathecal injection of dextrorphan. Each dextrorphan-treated group had a small group of control animals (n = 5 each), which were given an equal amount of normothermic (24°C) saline solution for 30 minutes after clamping. The rabbits were anesthetized with intravenous sodium pentobarbital (25 mg/kg). Intravenous vecuronium bromide (0.1 mg/kg) was also used in group E as a muscle relaxant to avoid movement during needle insertion into the spinal canal. The animals were allowed to breathe spontaneously except in group E, in which tracheotomy and mechanical ventilation were necessitated by use of the muscle relaxant. All groups received 0.5% lidocaine hydrochloride at the site of skin incision as local anesthesia. After the incision had been made in the neck, an arterial line was secured in the left common carotid artery with a 24-gauge catheter for monitoring of the arterial pressure proximal to the site of clamping. A continuous intravenous infusion line was secured in the left external jugular vein with a 24-gauge catheter. A continuous intra-aortic infusion line was established in the right femoral artery with a 20-gauge catheter, the tip of which extended to the infrarenal abdominal aorta. Rectal temperature was continuously monitored with a flexible probe (Terumo Medical Corporation, Tokyo, Japan). The animal was placed in the supine position and the infrarenal abdominal aorta was exposed through a transperitoneal approach. After intravenous or intra-aortic administration of heparin (100 U/kg), spinal cord ischemia was induced by cross-clamping the abdominal aorta just distal to the origin of the renal arteries and just proximal to the aortic bifurcation. Each of the 5 groups was subdivided into a number of separate cohorts on the basis of the duration of clamping time. The subgroups, which consisted of 5 animals each, were constructed in the following way: group A, at 20, 25, and 30 minutes of clamping; group B, every 5 minutes from 30 to 45 minutes of clamping; group C, every 5 minutes from 30 to 60 minutes of clamping; group D, every 5 minutes from 30 to 50 minutes of clamping; and group E, at 25, 30, and 40 minutes of clamping.

Drug Delivery

Dextrorphan (Hoffman-La Roche Inc, Basel, Switzerland) was dissolved in saline solution at a concentration of 10 mg/mL for the treat-
ment of groups B, C, and D. For group E, a 1-mg/mL concentration of dextrorphan was prepared. In group B, dextrorphan pretreatment (10 mg/kg) was done through the external jugular vein catheter 5 minutes before aortic clamping. This was followed by continuous intravenous infusion of dextrorphan at a rate of 1 mg/min with an infusion pump (Terumo) until 15 minutes after unclamping. Group C received continuous intra-aortic infusion of dextrorphan into the closed segment of the abdominal aorta after pretreatment (10 mg/kg) through the catheter in the right femoral artery 5 minutes before aortic clamping. Group D had similar dextrorphan pretreatment to group C and then received a bolus injection of dextrorphan (1 mg per minute of clamping time) into the closed segment of the aorta through the same route as group C immediately after clamping, as well as an additional 15 mg of dextrorphan after unclamping. In group E, a tracheotomy was done to start mechanical ventilation. Then laminectomy was performed via a posterior approach in the left lateral position. The spinous processes of L5 were removed, and the surrounding structures were dissected until the epidural fat was exposed. A bolus dose of dextrorphan (0.2 mg/kg) was given intrathecally 30 minutes before spinal cord ischemia was induced (Figure 1).

Neurologic Evaluation
Neurologic status was scored by assessment of hind-limb motor function 48 hours after unclamping according to the Johnson neurologic recovery scale: 0 = hind-limb paralysis; 1 = severe paraparesis; 2 = functional movement, no hopping; 3 = ataxic hopping; 4 = minimal ataxia; 5 = normal.

Histopathologic Examination
After these assessments, animals were put to death with an overdose of intravenous sodium pentobarbital. The lumbar spinal cord (L4 to L6) was quickly removed and fixed overnight in 10% buffered formalin. Paraffin-embedded spinal cord sections were processed for hematoxylin and eosin staining, and histopathologic evaluation, including a count of the remaining neurons per section in the gray matter, was performed under a light microscope.

Statistical Analysis
The average neurologic recovery score was calculated and was expressed as mean ± standard deviation (SD). Statistical analysis of the neurologic recovery scores was performed with the non-
Comparison of Average Scores

Each dextrorphan-treated group showed better neurologic function than their respective control group of animals after 30 minutes of clamping ($P < .001$ vs groups B, C, and D; $P = .014$ vs group E). Groups B, C, and D showed better neurologic function than group E ($P = .017$ vs group B; $P < .001$ vs group C; $P = .014$ vs group E) after 45 minutes of clamping. Furthermore, group C showed better neurologic function than group D after 50 minutes of clamping ($P = .017$). Therefore, among the dextrorphan-treated animals, those from group C showed the best neurologic function, and continuous intracranial infusion of dextrorphan prolonged the safe aortic clamping time to 55 minutes (Figure 3).

Histopathologic Evaluation

The spinal cords of animals that recovered fully (score 5) after dextrorphan treatment were histologically well preserved with normal-looking motor neurons, which had clear nucleoli and Nissl substance on hematoxylin and eosin staining of cross sections of the lumbar spinal cord. On the other hand, some spinal cords from the animals with hindlimb paralysis (score 0) demonstrated necrotic changes, including destruction and vacuolization of the gray matter, pyknosis of neurons, and eosinophilic changes of the cytoplasm, but others appeared almost normal. However, it was difficult to detect any obvious differences in the histologic features of spinal cords with scores between 2 and 4 (Figure 4). The counts of remaining neurons apparently corresponded to the average neurologic scores (Figure 5).

Discussion

Ischemic neuronal death in the spinal cord and in the brain occurs because excessive glutamate release activates the glutamate NMDA receptor and triggers intracellular Ca$^{2+}$ influx. The efficacy of noncompetitive NMDA receptor antagonists for prevention of neuronal ischemia has been documented, especially in the brain. Similar studies on the spinal cord have also been conducted recently through intravenous, intra-aortic, and intraperitoneal routes.

Dextrorphan, the $O$-demethylated metabolite of the common antitussive dextromethorphan, is an NMDA antagonist that attenuates hypoxic neuronal injury in cultured cells and significantly reduces ischemic neuronal injury in animal
stroke models. Pharmacokinetic studies have indicated that intravenous dextrorphan has a mean half-life of 1.7 to 5.4 hours. Dextromethorphan is rapidly converted to dextrorphan, which is 5 times more effective against hypoxic neuronal injury. In this study, dextorphan was administered by means of 4 different methods: continuous intravenous infusion, continuous intra-aortic infusion, bolus intra-aortic injection, and bolus intrathecal injection. Each of the 5 groups was divided into separate cohorts according to their duration of clamping. At first, neurologic outcome at 30 minutes of clamping was evaluated and then clamping time was gradually lengthened at an increment of 5 minutes (group B, C, and D) or 10 minutes at a time (group E) until the neurologic status of the animals clearly deteriorated. Control animals in all groups except group A became paraplegic at 30 minutes of clamping. It was observed that the dextorphan-treated animals of groups B, C, and D could safely withstand crossclamping for up to 30 minutes, whereas this duration for group E was 25 minutes. In our study, dextorphan was effective irrespective of the method of administration when compared with control animals. Continuous intra-aortic infusion was found to be the most effective method, and it prolonged the safe clamping time up to 55 minutes. Bolus intra-aortic injection was found to be a little more effective than continuous intravenous infusion. The reason for this efficacy probably is that intra-aortic dextorphan is delivered more effectively to the ischemic spinal cord. However, the extent of neurologic recovery showed some variation in both groups. Continuous intra-aortic infusion led to a better outcome than bolus intra-aortic injection, possibly because most of the dextorphan injected in one shot was washed away from spinal cord.

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<th>Clamping time (min)</th>
<th>Before</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
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<td>Group A</td>
<td>91 ± 6</td>
<td>90 ± 8</td>
<td>88 ± 8</td>
<td>87 ± 9</td>
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<td>Group B</td>
<td>94 ± 11</td>
<td>94 ± 9</td>
<td>85 ± 9</td>
<td>85 ± 8</td>
<td>89 ± 9</td>
<td></td>
<td></td>
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<tr>
<td>Group C</td>
<td>92 ± 8</td>
<td>89 ± 10</td>
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<td>90 ± 10</td>
<td>90 ± 7</td>
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<td>95 ± 11</td>
<td>89 ± 11</td>
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<td>85 ± 11</td>
<td>82 ± 12</td>
<td>89 ± 14</td>
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<td>Group E</td>
<td>89 ± 12</td>
<td>85 ± 13</td>
<td>85 ± 11</td>
<td>75 ± 9*</td>
<td>77 ± 13†</td>
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Values represent mean ± standard deviation. Group A (n = 15) received simple infrarenal aortic clamping; group B (n = 20), continuous intravenous infusion of dextorphan; group C (n = 35), continuous intra-aortic infusion of dextorphan; group D (n = 25), bolus intra-aortic injection of dextorphan; and group E (n = 15), bolus intrathecal injection of dextorphan.

*P < .05 versus groups A and D; †P < .001 versus group C.
intrathecal injection in rats. Several studies have been performed to prolong the safe clamping time, but it is difficult to insert an intrathecal catheter. Continuous intrathecal infusion of dextrorphan may infiltrate the entire spinal cord more effectively through the blood vessels than through the spinal fluid. Dextrorphan easily passes through the blood-brain barrier, and the blood level correlates well with the free brain level and with the extent of protection against ischemic neuronal damage. Thus, dextrorphan may infilitrate the entire spinal cord more effectively through the blood vessels than through the spinal fluid. Continuous intrathecal infusion of dextrorphan may prolong the safe clamping time, but it is difficult to insert an intrathecal catheter. Several studies have been performed with regard to percutaneous intrathecal injection in rats and rabbits. However, we found that percutaneous intrathecal injection in rabbits was difficult because the intrathecal space is very narrow. Blood pressure was stable in all groups while the aorta was crossclamped.

Hypothermia can inhibit glutamate release and prolong tolerance of ischemia. In this study, ischemia was induced with normothermia. The temperature of all groups except for the intrathecal group decreased spontaneously but was not significantly lower than that of group A. Therefore, hypothermia, which may have influenced the duration of the safe clamping time, did not play a major role in protecting the spinal cord from ischemia. Although the body temperature of the intrathecal group was significantly lower than in other groups, this may be attributable to the preischemic fall of temperature in this group. The reason for the preischemic hypothermia was that the preischemic operating time was longer in the intrathecal group because of additional invasive procedures. However, the rate of decrease in body temperature in this group was similar to that in the other groups.

Histopathologic findings did not necessarily correspond well with hind-limb neurologic function. Even the spinal cords of paraplegic animals were sometimes histologically normal. These results suggest that the mechanism of motor neuron deficits is complex and that the spinal cords of paraplegic animals do not always show obvious necrosis. We counted only the remaining neurons because necrotic neurons, which lost nuclear hematoxylin, were difficult to distinguish from surrounding tissue. Ortiz and associates described cytopathologic changes in the cingulate cortex of rats treated with a 30-mg/kg dose of dextrorphan, and these animals did not exhibit overt changes such as ataxia. The present study, however, revealed no obvious histopathologic changes in the animals that did not show any neurologic deficits.

It has also been reported that transient and reversible adverse effects, including nystagmus, nausea, vomiting, somnolence, hallucinations, agitation, and hypotension at high doses, can occur in dextrorphan-treated patients with acute stroke. However, such adverse effects resolve rapidly after discontinuation of infusion because of the short half-life of the drug, and dextrorphan does not exhibit any cardiac or other systemic toxicities.

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distributed systemically except in the crossclamped aortic segment. Therefore, intravenous dextrophan infusion is also expected to prolong the safe clamping time. A thorough application of our experimental procedure of continuous intra-aortic infusion to the thoracoabdominal operation in human beings is impossible. However, for the clinical use of this continuous intra-aortic infusion method, selective intercostal arterial perfusion, either through a small Dacron prosthesis or through catheters of 1-mm or 1.5-mm diameter, might be helpful in achieving continuous infusion directly into the intercostal arteries of the crossclamped aortic segment. However, insertion of such narrow catheters is often time consuming, technically difficult, and associated with risk of injury to the intercostal arteries. Continuous intrathecal infusion via a catheter was impossible in rabbits, but such intrathecal catheterization can be done percutaneously in human beings. The procedure is likely to be more effective if two catheters are used, one for infusion and the other for drainage.

The safe clamping time was prolonged in all of the dextrophan-treated animals when compared with the control animals. Continuous intra-aortic infusion of dextrophan, in particular, was able to prolong the safe aortic clamping time, which was significantly longer than that provided by any other route. The present experiment was done in an infrarenal aortic clamping model rather than a thoracoabdominal aortic clamping model. However, the results of this study suggest that dextrophan has the potential to reduce spinal cord ischemia in patients undergoing thoracoabdominal aortic operations. Further clinical investigations are required to determine the beneficial effects of dextrophan.

References