Role of \(\text{N-methyl-}d\text{-aspartate}\) receptor in acute spinal cord injury

MITSUHIRO YANASE, M.D., TAKASHI SAKOU, M.D., AND TAKEO FUKUDA, M.D.

Departments of Orthopedic Surgery and Pharmacology, Kagoshima University, Faculty of Medicine, Kagoshima, Japan

To clarify the role of \(\text{N-methyl-d-aspartate}\) (NMDA) receptors in acute spinal cord injury, changes in the intraspinal microcirculation after acute spinal cord injury in rabbits were examined. Systemic administration of MK-801, an NMDA receptor antagonist, at a dose of 5 mg/kg, significantly improved motor recovery after injury and significantly reduced edema formation at the injured site without altering spinal cord blood flow or vascular permeability at the injured site. These findings indicate that excitatory amino acids contribute to secondary spinal cord damage, especially edema formation, mediated by NMDA receptors in the early stage after injury.

**KEY WORDS** • \(\text{N-methyl-}d\text{-aspartate}\) receptor antagonist • edema • spinal cord injury • spinal cord blood flow • vascular permeability • rabbit

O f the numerous experimental studies that have been conducted to examine pathophysiological changes after acute spinal cord injury, most are consistent with a two-step damage mechanism of the spinal cord, in which the primary or mechanical injury is caused by an external force and secondary or progressive autodestructive injury of the cord follows.\(^1\) Factors believed to play a significant role in the progressive post-traumatic autodestruction include changes in spinal microcirculation,\(^2\) norepinephrine release,\(^18\) cell membrane damage with lipid hydrolysis,\(^4\) production of arachidonic acids and free radicals, accumulation of dynorphin, changes in the tissue content of monovalent and divalent metal cations,\(^9\) and release of excitatory amino acids mediated by \(\text{N-methyl-}d\text{-aspartate}\) (NMDA) receptors.\(^{10,11,24}\)

The effects of excitatory amino acid release, mediated by an NMDA receptor, on changes in the spinal microcirculation after acute spinal cord injury is attracting interest, but the details remain unknown. In this study we administered MK-801, an NMDA receptor antagonist,\(^{27}\) to rabbits with experimental spinal cord injury to examine the action of NMDA receptors in the posttraumatic autodestruction.

**Materials and Methods**

**Experimental Model Preparation**

Seventy-four Japanese albino rabbits, each weighing between 1.7 and 2.2 kg, were used in this study. Endotracheal intubation was performed via tracheotomy after intravenous administration of sodium pentobarbital (40 mg/kg). Blood gas levels and arterial blood pressure were monitored through a femoral artery catheter, and drugs were administered through an ear-vein catheter. The rabbits were immobilized with continuous intravenous administration of pancuronium bromide (0.02 mg/kg/hr), then mounted on a stereotactic frame. General anesthesia was induced with nitrous oxide gas. The PaO\(_2\) level was maintained at 90 to 120 mm Hg and PaCO\(_2\) at 35 to 45 mm Hg with a respirator.

Laminectomy was performed at the T5–8 level. One myelomere of the T-8 segment was compressed by placing a 50-g square compressor on the dura mater over it for 1 minute.\(^{22,30}\) This technique creates a model of spinal cord injury that causes paralysis of the lower extremities in a reproducible manner. The injured rabbits were randomly assigned to receive either 1.0 ml/kg of saline solution containing MK-801 (total dose 5 mg/kg) or an equal volume of physiological saline solution. The solutions were administered through the ear-vein catheter 10 minutes after compression.

**Evaluation of Neurological Recovery**

Neurological recovery was evaluated daily for 2 weeks after injury using the Tarlov and Klinger\(^23\) ordinal scale based on motor function. According to the scale, 0 = complete paraplegia, 1 = some minor movement of the joints, 2 = major movement of the joints but inability to stand, 3 = the animal can stand and possibly walk, and 4 = the animal can run and has a normal motor system with no obvious weakness.

**Posttrauma Measurements**

Regional spinal cord blood flow (SCBF) was determined for 6 hours using a laser Doppler flowmeter (Periflux PF3; Perimed, Stockholm, Sweden), which expresses SCBF as a perfusion index. The tips of the laser Doppler needle probe were continuously placed on the dura mater at the T-5 and T-8 segments.
Role of NMDA receptor in spinal cord injury

To obtain the water content of the spinal cord, the spinal cord parenchyma at T-5 and T-8 was excised with a knife. Each section was weighed immediately, then reweighed after complete drying at 110°C for 3 hours in a heating chamber. The water content was expressed as a percentage.

The vascular permeability of the spinal cord was evaluated by measuring the intraspinal concentration of intravenous fluorescein isothiocyanate dextrans (FD 70S) dissolved in distilled water (25 mg/ml), administered at a dose of 50 mg/kg. Sections of spinal cord at the T-5 and T-8 vertebral levels were excised 10 minutes after injection and homogenized with 19 volumes of saline. The homogenized solution (1 ml) was allowed to react with 1 N HClO4 (0.5 ml) and was centrifuged at 16,000 rpm for 20 minutes. The supernatant (200 μl) was reacted with barbital buffer (3 ml), and the concentration of fluorescent dye was determined with the aid of a spectrophotofluorimeter (MPK-4K; Hitachi, Tokyo, Japan) at an excitation wavelength of 492 nm and an emission wavelength of 516 nm. The fluorescent dye concentration was expressed as microgram per gram.

Statistical Analysis

Results were given as the mean ± standard error of the mean. The data were analyzed with the unpaired two-tailed t-test. Differences with a probability value of less than 0.05 were considered to be statistically significant.

Results

Arterial Blood Pressure

Rabbits treated with both saline and MK-801 showed a rapid increase in mean arterial blood pressure up to 150 ± 12 mm Hg during spinal compression, followed by a sharp decline. The blood pressure was reduced throughout the 6-hour measurement period. All seven rabbits treated with MK-801 showed a lower blood pressure than the seven controls, although this difference was significant (p < 0.01) only during the 1st hour after injury (Fig. 1).

Neurological Recovery

The neurological scores of the MK-801–treated rabbits studied were significantly higher than those of the controls at 24 hours after injury (p < 0.01). Three days after injury, all 20 animals in the MK-801 treatment group showed complete recovery from paralysis. In the physiological saline group, no animals showed complete recovery at 3 days, and 2 weeks was required for all 20 animals to recover completely from paralysis (Fig. 2).

Spinal Cord Blood Flow

Regional SCBF at the T-8 segment decreased gradually after injury in both treatment groups (seven rabbits each), whereas no decrease was observed at the T-5 segment. There was no significant difference between the groups in regional SCBF (Fig. 3).

Water Content of the Spinal Cord

The water content of the spinal cord was found to be 67.2% ± 0.11% in normal rabbits. After injury, the water content at the T-8 segment showed a rapid increase in the seven control rabbits (mean value 1 hour after compression 69.8% ± 0.58%) and remained elevated for the first 24 hours after injury. No change was seen at the T-5 segment. In contrast, the increase in water content after injury was less marked in the seven MK-801–treated rabbits, especially at 1 hour (mean value 67.57% ± 0.37%, p < 0.01). There was also a significant difference in water content between the groups in water content 6 hours after injury (p < 0.05) (Fig. 4). No increase in water content was seen at the T-5 segment in either group.

Fig. 1. Graph showing changes in mean arterial blood pressure (MABP) in the two groups of rabbits. Values are expressed as means ± standard error of the means for two experiments each. Statistical significance: ** = p < 0.01 (saline-treated control vs. MK-801–treated group).

Fig. 2. Graph depicting neurological scores in the two groups of rabbits. Grading according to Tarlov and Klinger. Values are expressed as means ± standard error of the means for two experiments each. Statistical significance: * = p < 0.01 (saline-treated control vs. MK-801–treated group).
Intraspinal Vascular Permeability

Examination of the intraspinal vascular permeability showed no marked difference was observed between the treated and control rabbits in the intraspinal concentration of FD 70S, although the treated group showed a lower concentration at the T-8 cord segment 1 hour after injury. The FD 70S concentration at the T-5 segment was low and within the normal range in both groups of seven rabbits (Fig. 5).

Discussion

Endogenously released excitatory amino acids have a neurotoxic effect on the impaired central nervous system, as in cases of ischemia and hypoglycemia.\(^{17}\) The presence of postsynaptic receptors for these amino acids in the nervous system has been confirmed. The receptors have been classified on the basis of preferred agonists determined by electrophysiological studies as: NMDA, quisqualate, and kainate receptors.\(^{25}\) The NMDA receptors are prominent in the brain cortex and spinal cord and are thought to play a major role in neurotoxic damage in the nervous tissues.\(^{19,21,26}\) However, the mechanism of neuronal tissue damage has not been established in in vivo studies. Two mechanisms of neuronal injury based on the time course and cellular ionic alterations observed in in vitro studies have been suggested: namely, early intracellular accumulation of sodium and chloride ions resulting in acute cytotoxic edema and intracellular influx of extracellular calcium ions resulting in delayed neuronal damage.\(^{5,14}\)

Recently, it has been suggested that NMDA receptors may play a major role in the secondary neuronal damage after traumatic spinal cord injury.\(^{6,9,11,18,16,24}\) Faden and associates\(^{10,11}\) observed that NMDA applied directly to the injury site exacerbated paralysis in rats, and NMDA antagonist administration resulted in significant neurological recovery. In the present study, treatment with the NMDA antagonist MK-801 resulted in significantly earlier recovery from motor paralysis in rabbits with incomplete spinal cord injury. This finding is in agreement with that reported by Faden and associates.

The decrease in regional blood flow of the spinal cord at the injury site begins immediately after injury and continues for several hours.\(^{8,24}\) There have been no studies of regional SCBF during and after NMDA antagonist administration. In this study, no improvement in the regional SCBF was observed: it was reduced in both the treated and untreated rabbits. In our laboratory, the hydrogen clearance method was previously used to measure regional SCBF;\(^{22,30}\) however, in recent years we have often used laser Doppler flowmetry to assess regional blood flow in vivo. This method, which was adopted in the present study, has an advantage in that it allows the evaluation of relative changes in regional blood flow over time, although it does not measure absolute blood flow.

We have already established a method of measuring the concentration of sodium fluorescein, which is administered systemically to assess the vascular permeability of experimentally injured spinal cords.\(^{22,30}\) When compared with the conventional histological techniques,\(^{28}\) this method permits quantitative analysis of the vascular permeability of the injured spinal cord and also has higher reproducibility and reliability. In the present study, we used FD 70S, with a higher molecular weight than sodium fluorescein. The use of FD 70S allowed us to evaluate intraspinal vascular permeability more precisely than with sodium fluorescein. When evaluated using FD 70S, the intraspinal vascular permeability was enhanced immediately after spinal cord injury but had nearly returned to normal by 6 hours after injury. This course of intraspinal vascular per-
meability was similar to that previously observed using sodium fluorescein. The administration of the NMDA antagonist did not significantly suppress the vascular permeability of the injured spinal cords, but it did suppress permeability slightly 1 hour after injury.

Yashon, et al., found that spinal edema at the injury site in rats developed immediately after injury and continued for 24 hours. In our study, the MK-801-treated group showed less edema in the early stage after injury but it increased after that.

The present findings indicate that the NMDA antagonist MK-801 is effective both in repairing motor impairment and in reducing spinal edema after acute traumatic spinal cord injury. Although vascular changes have been cited as one of the important factors in the secondary destruction of the injured spinal cord, no significant effects of the NMDA antagonist on SCBF or vascular permeability were detected in this study.

Conclusions

The NMDA antagonist MK-801 was demonstrated to have a significant effect on recovery from motor impairment after acute spinal cord injury in rabbits. However, the vascular change parameters of SCBF and the vascular permeability in this spinal cord injury model were not significantly influenced by administration of the NMDA antagonist. On the basis of these findings, we consider that the excitatory amino acids at the injured site act directly on the surviving nerve cells to promote their destruction by inducing cellular ionic alterations resulting in cytotoxic edema.

Acknowledgments

The authors wish to thank Takao Shimizu, Ph.D., Kagoshima University, for his helpful assistance. We also thank Asaka Shimofuku for secretarial assistance.

References


Manuscript received July 14, 1993.
Accepted in final form January 5, 1995.
This study was supported in part by Grant 01480372 from the Monbusho Scientific Research Fund.
Address reprint requests to: Mitsuhiro Yanase, M.D., Depart-ment of Orthopedic Surgery, Kagoshima University, 8-35-1, Saku-ragaoka, Kagoshima 890, Japan.