Evaluation of the Neuroprotective Effect of Minocycline in a Rabbit Spinal Cord Ischemia Model

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Objective: **To investigate whether postischemic administration of minocycline attenuates hind-limb motor dysfunction and gray and white matter injuries after spinal cord ischemia.**

Design: **A prospective, randomized, laboratory investigation.**

Setting: **Laboratory in university, single institution.**

Participants: **Male New Zealand White rabbits.**

Intervention: **Spinal cord ischemia was induced by an occlusion of the infrarenal aorta for 15 minutes. The groups were administered minocycline 1 hour after reperfusion (M-1; n = 8), minocycline 3 hours after reperfusion (M-3; n = 8), saline 1 hour after reperfusion (control [C]; n = 8), or saline and no occlusion (sham; n = 4). Minocycline was administered intravenously at 10 mg/kg 6 times at 12-hour intervals until 60 hours after the initial administration.**

Measurement and Main Results: **Hind-limb motor function was assessed using the Tarlov score. For histologic assessments, gray and white matter injuries were evaluated 72 hours after reperfusion using the number of normal neurons and the percentage of areas of vacuolation, respectively. Motor function 72 hours after reperfusion was significantly better in group M-1 than in group C. The number of neurons in the anterior horn was significantly larger in group M-1 than in groups M-3 or C but did not differ significantly between groups M-3 and C. No significant difference was noted in the percentage of areas of vacuolation among the ischemia groups.**

Conclusions: **Minocycline administration beginning at 1 hour after reperfusion improved hind-limb motor dysfunction and attenuated gray matter injury in a rabbit spinal cord ischemia model.**

KEY WORDS: minocycline, ischemic spinal cord injury, reperfusion, neuroprotective effect, rabbit

AN IMPORTANT COMPLICATION of thoracic or thoracoabdominal aortic aneurysm is lower-limb paralysis owing to spinal cord ischemia (SCI).[1,2] Hypothermia therapy, spinal cord drainage, and various drug therapies to protect the spinal cord from ischemia have been reported,[3,4] but their effects were limited. Therefore, further efforts to clarify the mechanisms of nerve injury and develop neuroprotective drugs are being made.

Minocycline is a semisynthetic tetracycline antibiotic that passes the blood-brain barrier and enters the central nervous system and is noted for its biologic rather than antibiotic actions.[5-8] It has been reported to decrease the size of ischemic foci in brain ischemia models.[9-12] Based on these reports, an open-label trial conducted in patients with acute stroke showed a more favorable outcome compared with the placebo group.[13] In spinal cord injury models, minocycline has been shown to alleviate the impairment of spinal cord tissue and improve motor functions in contusion and compression models.[14-17]

The authors' previous study indicated that the intraperitoneal administration of minocycline before ischemia improved the hind-limb motor function and alleviated the impairment of the gray and white matters in a rat SCI model.[18] However, there are no data available on minocycline-mediated neuroprotection when administered after reperfusion in a model of SCI. The present study was conducted to examine whether minocycline administration after reperfusion also had a neuroprotective effect in a rabbit model of SCI and to evaluate the therapeutic period of this effect.

METHODS

This study was approved by the animal experiment committee of Nara Medical University (Kashihara, Nara, Japan). Twenty-eight New Zealand White rabbits weighing 2.7-3.0 kg were used. These rabbits were housed under 12-hour light-dark cycles with free access to food and water.

The rabbits were allocated randomly to 1 of the following 4 groups: a control (C) group (n = 8), a group administered minocycline 1 hour after reperfusion (M-1; n = 8), a group administered minocycline 3 hours after reperfusion (M-3; n = 8), or a sham group (n = 4). In the M-1 and C groups, minocycline (Nichi-iko, Toyama, Japan; M-1 group) or normal saline (C group) was administered intravenously every 12 hours from 1 hour after reperfusion for 60 hours (total, 6 times). In the M-3 group, minocycline was administered every 12 hours for 60 hours, but the administration was started 3 hours after reperfusion. The dose of minocycline was 10 mg/kg (in 3 mL). The sham group was administered the same amount of normal saline at the same schedule as in the C group and did not undergo aortic occlusion.

The rabbits were anesthetized in a plastic box using oxygen and 5% isoflurane. A catheter was inserted into the auricular vein, and Ringer's solution was administered at 10 mL/kg/h. The auricular artery was cannulated, and the proximal arterial pressure was measured. After the intravenous administration of fentanyl at 50 μg/kg, endotracheal intubation was carried out, and artificial ventilation (Harvard Respirator 510, Summit Medical, Worcester, MA) was performed using 2%-3.5% isoflurane, 30% oxygen, and air by adjusting the end-tidal carbon dioxide pressure to 35-40 mmHg. The rectal temperature was monitored continuously, and it was adjusted to 38-39°C using an electric mat. For measurement of the distal arterial pressure and blood sampling (blood gas and blood sugar analyses), the right femoral artery was exposed, and an SP-55 catheter was inserted. A 50-mm flank incision at the left costal level was made after the infiltration of 1% lidocaine, and the aorta was exposed from the retroperitoneal cavity at the left renal artery level. A silicone thread, 1.5-mm wide, was placed carefully around the aorta immediately distal to the left renal artery, and the 2 ends of the thread were passed through an occluder tube. Before the induction of ischemia, heparin, 600 U, was administered. The aorta was

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This work was supported by Grant-in Aid for Scientific Research C2-22591715 (Japan).

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1053-0770/2606-0011$36.00/0

http://dx.doi.org/10.1053/j.jvca.2012.05.003

Table 1. Physiologic Variables

	Sham (n = 4)	C (n = 8)	M-1 (n = 8)	M-3 (n = 8)
Weight (kg)	2.9 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	2.9 ± 0.1
Mean arterial pressure (mmHg)				
Before	67 ± 7	63 ± 5	60 ± 11	65 ± 7
During	66 ± 10	12 ± 2*	12 ± 1*	12 ± 1*
After	ND	54 ± 10	52 ± 7	57 ± 4
Rectal temperature (°C)				
Before	38.7 ± 0.2	38.6 ± 0.4	38.5 ± 0.3	38.6 ± 0.3
During	38.3 ± 0.3	38.7 ± 0.3	38.5 ± 0.2	38.7 ± 0.3
After	38.4 ± 0.4	38.6 ± 0.3	38.7 ± 0.3	38.5 ± 0.2

NOTE. Data are expressed as mean ± standard deviation. Mean arterial pressure and rectal temperature were measured 10 minutes before aortic occlusion, during ischemia (7.5 min after aortic occlusion), and 10 minutes after reperfusion.

Abbreviations: C, control; M-1, minocycline administered 1 hour after reperfusion; M-3, minocycline administered 3 hours after reperfusion; ND, no data.

*$p < 0.05$ versus sham.

occluded by tightening the thread using the tube, and the occlusion was confirmed by monitoring the distal pressure. After 15 minutes, the aortic occlusion was released. After reperfusion, all catheters were removed and the wound was closed. Blood was sampled 10 minutes before the aortic occlusion and 10 minutes after reperfusion. The blood pressure and body temperature were measured 10 minutes before the aortic occlusion, during the ischemia (7.5 min after aortic occlusion), and 10 minutes after reperfusion.

The hind limbs of the rabbits were evaluated neurologically 24, 48, and 72 hours after reperfusion by a blinded observer. Assessment was made using Tarlov scoring,[19] which consists of a 5-point grading scale (0, paraplegic with no lower extremity function; 1, poor lower extremity function, weak antigravity movement only; 2, some lower extremity motor function with good antigravity strength but inability to draw leg under the body or hop; 3, ability to draw legs under the body and hop but not normally; 4, normal motor function).

Following the neurologic evaluation after 72 hours, thiopental, 50 mg/kg, was administered intraperitoneally, and the animals were anesthetized deeply using 5% isoflurane. After 1,000 mL of cold heparinized physiologic saline was infused transcardially, 500 mL of 4% paraformaldehyde in phosphate buffered saline, 0.1 mol/L, was administered. The lumbar spinal cord was removed and immersed in paraformaldehyde for 2 days, and the L5 part was cut off, embedded in paraffin, and sliced at a thickness of 3 μm for hematoxylin and eosin staining.

Gray matter injury was evaluated by the number of normal neurons remaining in the left anterior horn of the spinal cord. An observer not informed of the randomization results counted the normal neurons in an area of the left hemisphere anterior to the central canal of the spinal cord under light microscopy (×200). White matter injury was evaluated by examining the ventral, ventrolateral, and lateral areas and calculating the percentage of areas of vacuolation, consisting of the percentage of a 0.04-mm^2 area occupied by vacuoles. Each area was divided into 144 subareas using grid lines; a subarea was regarded as a vacuolated area if ≥75% of it was occupied by vacuoles, and the percentage of vacuolated subareas in the 144 subareas was calculated. The total percentage of areas of vacuolation was calculated as a mean of the 3 areas on each side.

The physiologic variables, the number of normal neurons, and the percentage of areas of vacuolation were analyzed using 1-factor analysis of variance, and the Fisher Protected Least Significant Difference (PLSD) was used as a post hoc test. The motor function of the hind limbs was examined with the Kruskal-Wallis test, and the Mann-Whitney *U*-test was used as a post hoc test. A p value <0.05 was considered statistically significant.

RESULTS

The physiologic variables are listed in Table 1. There were no significant differences in body weight or rectal temperature among the groups. Blood pressure values were similar among the groups before aortic occlusion but were significantly higher in the sham group than the ischemic groups during ischemia. Blood gas data are presented in Table 2. No significant difference was noted in any of the blood test items, including the pH, arterial partial pressures of carbon dioxide and oxygen, hematocrit, and blood glucose levels among the groups.

Tarlov scores 24, 48, and 72 hours after reperfusion are listed in Table 3. Figure 1 shows the individual neurologic scores 72 hours after reperfusion. Tarlov scores in the sham group were significantly higher compared with those in the 3 groups exposed to ischemia. Tarlov scores 72 hours after reperfusion

Table 2. Blood Gas Data

	Sham (n = 4)	C (n = 8)	M-1 (n = 8)	M-3 (n = 8)
pH				
Before	7.49 ± 0.04	7.46 ± 0.05	7.50 ± 0.07	7.4 ± 0.05
After	7.46 ± 0.01	7.44 ± 0.06	7.45 ± 0.05	7.4 ± 0.04
$PaCO_2$ (mmHg)				
Before	37 ± 4	38 ± 3	36 ± 3	37 ± 4
After	30 ± 3	36 ± 6	35 ± 4	35 ± 3
PaO_2 (mmHg)				
Before	160 ± 44	159 ± 31	154 ± 30	134 ± 30
After	195 ± 16	171 ± 33	164 ± 28	152 ± 23
Hematocrit (%)				
Before	36 ± 4	34 ± 2	35 ± 3	37 ± 3
After	33 ± 3	33 ± 2	33 ± 2	35 ± 3
Glucose (mg/dL)				
Before	116 ± 22	123 ± 18	137 ± 28	138 ± 23
After	151 ± 19	160 ± 45	139 ± 44	142 ± 32

NOTE. Data are expressed as mean ± standard deviation. Blood was sampled 10 minutes before aortic occlusion and 10 minutes after reperfusion. There were no significant differences among the groups.

Abbreviations: C, control; M-1, minocycline administered 1 hour after reperfusion; M-3, minocycline administered 3 hours after reperfusion; $PaCO_2$, arterial partial pressure of carbon dioxide; PaO_2, arterial partial pressure of oxygen.