The Neuroprotective Effect Of Erythropoietin On Motor Neurons In The Spinal Ventral Horn After Nerve Root Avulsion Injury In Rats

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Disclosures:

Introduction: Total brachial plexus avulsion injury is one of the difficult-to-treat nerve injuries. Several treatments, such as nerve transfer, muscle transfer, tendon transfer and arthrodesis have shown limited and insufficient functional recovery due to the lack of donor nerves. A novel surgery involving re-implantation of the avulsed nerve root into the spinal cord has been reported. Although this procedure still has a number of unsolved shortcomings, limited functional recovery of the shoulder and elbow joints was seen in some cases. After nerve root avulsion injury, the number of motor neurons in the affected spinal cord segment reportedly decreases significantly within several weeks. Therefore, addressing motor neuron death is one of the therapeutic targets for nerve root avulsion injury. A hematopoietic hormone, erythropoietin (EPO), was reported to exert neuroprotective effects in some traumatic and degenerative diseases. In the present study, we investigated the neuroprotective effect of EPO on motor neurons in the spinal ventral horn after nerve root avulsion injury using a rat model.

Methods: Nine-week old, male Sprague-Dawley rats were used for our examinations. Under general anesthesia, nerve root avulsion surgery was performed in the rats. The left C6 spinal nerve root was avulsed from outside the cervical vertebra using a posterior approach. Forty rats were randomly divided into the following eight groups according to the time of first EPO administration after surgery (n = 5 each): control (saline administration), immediate, 6 h, 24 h, 48 h, 96 h, 150 h and 300 h. EPO (2680 unit/kg) was injected subcutaneously once a day for three days. At 28 days after the surgery, C6 spinal segments were removed following perfusion fixation with 4% PFA. Then, 40 µm-thick, frozen, serial transverse sections were made. The number of motor neurons in the ventral horns bilaterally was counted every two sections after Nissl staining. The number of surviving motor neurons on the side of the lesion was expressed as a percentage of those on the intact side (right side).

For other histological examinations, 26 operated rats were randomly divided into the following two groups (n = 13 each): control group (saline) and EPO-treated group. Administration of saline or EPO was commenced immediately after the surgery in both groups and EPO (2680 unit/kg) was injected subcutaneously once a day for three days. At 3 and 28 days after the surgery, 5 rats in each group were sacrificed and 40 µm-thick, frozen, serial transverse sections of the C6 segment were made. Then, the number of cells immunopositive for Iba-1 (microglia) and GAP-43 (neuron regeneration marker) were counted. The two markers indicate an inflammation after the surgery and an opportunity of re-implantation surgery, respectively. For assessment of the reactive oxygen species (ROS), 3 rats in each group were injected with hydroethidine (HEt) intravenously at one day after the surgery and then sacrificed. Ten µm-thick, frozen, serial transverse sections of the C6 segment were made and fluorescence analysis was performed. Another 6 operated rats without administration of EPO were used for investigation of the EPO receptor (EPOR). EPOR positive cells were assessed at 3, 7 and 14 days after the surgery (n = 2 each). All animal studies were conducted in accordance with the principles and procedures approved by Kyoto University Committee of Animal Resources. All data were expressed as the mean ± SEM. Dunnett’s test was used for statistical analysis of the first investigation and Student t-test was used for the other examinations. P < 0.05 was considered significant.

Results: EPO-treated rats showed significant improvement in the proportion of preserved motor neurons compared to control rats in animals in whom the initial dose was started within 96 h after the surgery (control: 47.9%, immediately: 74.2%, 6 h: 79%, 24 h: 77.6%, 48 h: 75.5%, 96 h: 73.9%, 150 h: 53.1%, 300 h: 51.7%, p < 0.01). Immunohistochemical examination showed that proliferation of microglia (Iba-1 positive cells) was significantly suppressed in EPO-treated rats compared to control rats at 3 days after injury (71.4 versus 136.7 per section, p < 0.01). At 28 days after injury, GAP-43 positive cells in the ventral horn of the spinal cord were significantly increased in EPO-treated rats compared to control rats (5.3 versus 1.3 per section, p < 0.01). In EPO-treated rats, ROS signals in the ventral horn were inhibited compared to those in control rats. EPOR were found on the bodies of motor neurons at every time point assessed.

Discussion: In contrast to spinal cord injury, nerve root avulsion causes only slight parenchymal injury in the spinal cord. However, death occurred in many motor neurons in the ventral horn within 4 weeks after the nerve root avulsion injury. This could be due to a variety of cytotoxic mechanisms evolving from minor primary mechanical damage, inducing the so-called secondary spinal cord injury, with further irreversible progression.

EPO reportedly has a neuroprotective effect after traumatic injury to the central nervous system. Our study showed that commencement of EPO administration within 96 hours after injury significantly improved the number of surviving motor neurons. In spinal cord injury, because of the large area of mechanical injury, neuroprotective agents should be administered as early as possible to reduce secondary damage. However, with root avulsion injury, due to the small amount of parenchymal
damage, free radical generation around motor neurons is likely to be limited. Therefore, even delayed administration of EPO for up to 4 days after the injury would be effective, as seen by the neuroprotective effect on motor neurons at 28 days after injury in rats in whom EPO administration was commenced up to 96 hrs. post-injury. Although EPORs were detected even 7 days post-injury, administration of EPO at this stage did not exert neuroprotective effects. This could be because an irreversible cascade of neuronal cell death might have already started. Microglia have been reported to release a variety of proinflammatory mediators that contribute to neuronal cell death in response to injury. In our study, microglial activation and ROS generation were inhibited by the administration of EPO. Although the detailed mechanism is still unclear, the neuroprotective effect of EPO after nerve root avulsion injury could be related to suppression of microglial proliferation and ROS generation.

**Significance:** This study showed neuroprotective effect of erythropoietin after the nerve root avulsion injury. The administration of EPO in the hyperacute phase may have a potential to develop the treatment of total brachial plexus avulsion injury.

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