Role of Rho-Associated Coiled-Coil Containing Protein Kinase in the Spinal Cord Injury Induced Neuropathic Pain

Kazuya Kishima[1], Masakazu Toi[2], Keishi Maruo[3], Fumihiro Arizumi[4], TOMOYUKI KUSUKAWA[5], Toshiya Tachibana[6]
[1]Hyogo College of Medecine, [2]Departmet of Orthopaedic Surgery, Hyogo Medical Un, [3]Departmet of Orthopaedic Surgery, Hyogo College of Medicine, [4]Department Orthopaedic Surgery, Hyogo College of M, [5]Department of Orthopaedic Surgery, Hyogo College o, [6]Department of Orthopaedic Surgery, Hyogo College of Medicine

INTRODUCTION:

Spinal cord injury (SCI) commonly results not only in motor paralysis but also in the emergence of neuropathic pain, both of which can impair the quality of life for SCI patients.

SCI can lead to increased phosphorylation of p38 in spinal cord microglia. This is one of the main causes for the development of persistent pain. Recently, we reported our study on the activation of p38 mitogen-activated protein kinases (MAPK) in spinal microglia, which has been considered the key molecule for the onset and maintenance of neuropathic pain after peripheral nerve injury, using a rat model. We also reported that the RhoA/Rho-associated coiled-coil containing protein kinase (ROCK) pathway mediates p38 activation in spinal microglia in peripheral nerve injury. But the precise mechanisms of neuropathic pain induced by SCI are still unclear. This study aimed to examine the activation of microglia and the p38 MAPK expression in the lumbar spinal cord after thoracic SCI in rats, and the correlation to the therapeutic effect of ROCK inhibitor ripasudil in rats with SCI.

METHODS:

Spinal cord contusion injury was produced in male Sprague–Dawley rats (180–200 g). The rats were anesthetized by three types of mixed intraperitoneal anesthetics. The lamina of the T10 vertebra was then removed. A contusion injury was generated using an impactor device that emulates a 100-kilodyne weight-drop onto the dura mater from a distance of 3-4 mm. Sham-controlled rats received laminectomy without the contusion injury. At several time points (0, 1, 3, 7, and 14 days) after the SCI, the groups of rats were processed for histological analysis (n=5 at each timepoint).

The Animal Care and Use Committee at Hyogo College of Medicine approved the experimental protocol, and the ethical guidelines of the International Association for the Study of Pain were adhered to in these experiments.

Rats were deeply anesthetized with three types of mixed intraperitoneal anesthetics and perfused transcardially. The spinal cord (L4–L5) was dissected out and postfixed in the same fixative at 4˚C overnight, followed by immersion in 20% sucrose in 0.1 M PB at 4˚C for 2 days. The tissue was frozen in powdered dry ice and cut on a cryostat at to a 16mm thickness for the spinal cord. The sections were processed for Immunohistochemistry. Rabbit-antiphospho-p38 MAPK (p-p38) polyclonal antiserum, goat antiionized calcium-binding adapter molecule 1 (Iba1) polyclonal antiserum and rabbit anti-P2Y12 polyclonal antiserum were used as the primary antibody. In brief, spinal cord sections were incubated with a primary antibody for 4 days at 4˚C. Double-immunofluorescent staining was performed with antirabbit Alexa Fluor 488 IgG, and anti goat Alexa Fluor 555 IgG after incubation with respective primary antibodies.

To quantify positive cells profiles in the spinal cord, laminas I–III was captured, and all of the positively stained cells in the area were counted with a computer-assisted imaging analysis system (NIH image).

Regarding drug treatment, the L5 vertebra was removed via laminectomy and a soft tube (outer diameter, 0.64 mm) filled with saline was inserted into the subarachnoid space just before SCI surgery. The mini-osmotic pumps filled with ROCK inhibitor ripasudil hydrochloride hydrate (24 nmol/day or 240 nmol/day) or saline were connected to the soft tube. Ripasudil and saline were intrathecally administrated from 0 to 3 days after SCI. For IHC, 3 days after the pump infusion, rats (n=3, each group) were perfusion fixation was performed and the spinal cord (L4–L5) was removed, and we compared them among the 3 groups using immunohistochemistry.

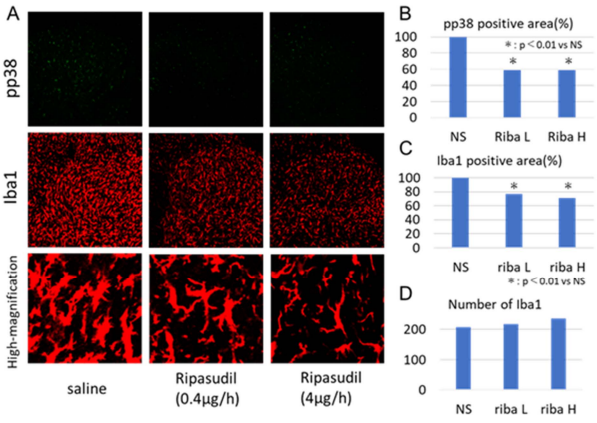
RESULTS:

The p-p38 positive cell and Iba1 (a maker of microglia) positive area ware increases at lumber spinal dorsal horn (L4-5) after 3 days and 7 days after SCI compared with the naive-control ($p<.05$) (fig.1A), whereas phosphorylated p38 was colocalized with microglia. (Fig 1B). Three days after SCI, microglia and p-p38 was significantly increased compared to naïve rats($p<.05$) (Fig 1C.D).

We injected the inhibitor, intrathecally into rats that received SCI. Three days after SCI, the intensity of phosphorylated p38 and Iba1 immunoreactive cells in the dorsal horn was significantly lower in the drug treated groups than saline group. And microglia got thinner and processed in the drug treated groups. (Fig2A-C). But administration of the inhibitor did not affect the numbers of microglia. (Fig2D).

DISCUSSION AND CONCLUSION:

Our results suggest that activation of ROCK in spinal cord microglia is likely to have an important role in the activation of p38 MAPK, which has been considered as a key molecule that switches on neuropathic pain after SCI. Inhibition of ROCK signaling may offer a means to develop a novel neuropathic pain treatment after SCI. It may help patients with neuropathic pain after SCI.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pp38 positive area(%)
＊: p＜0.01 vs NS
100
80
60
40
20
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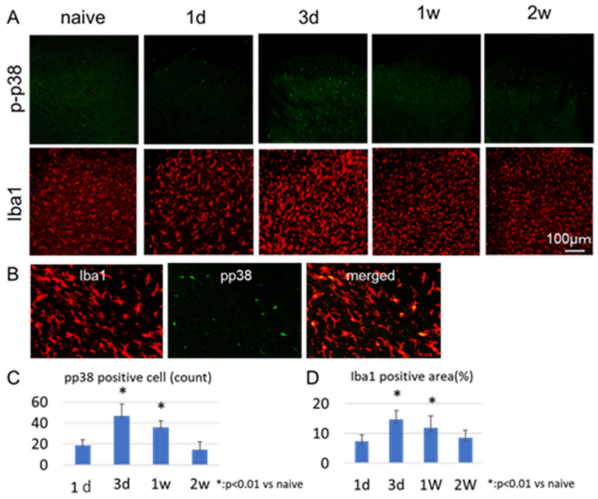
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Iba1 positive area(%)
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