p38 mitogen activated protein kinase inhibitor reduces the production of chondroitin sulfate proteoglycans after the spinal cord injury in rat

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Introduction
Spinal cord injury (SCI) is serious medical condition which induces permanent paresis or paralysis. Many trials to develop effective treatment for SCI have been done. However, there has been no successful treatment yet. Immediately after traumatic SCI, initial physical damage such as, bleeding in spinal tissue, rupturing axons occurs subsequently, secondary neuronal damages, including tissue necrosis, ischemia and inflammation, are followed around the damaged area from several hours to days after SCI. At 2-3 weeks after SCI, a cavity was formed as a result of necrosis/apoptosis in damaged spinal cord tissue. Glial scar, which inhibits axonal regeneration, is developed around the cavity. One of the inhibitory factors in the scar is chondroitin sulfate proteoglycans (CSPGs). CSPGs including neurocan, brevican, phosphacan and versican are produced by reactive astrocytes. Leonard et al. (1) reported that CSPGs were produced in injured area and extend in damaged spinal cord from one day to at least 8 weeks after SCI. Bradbury et al. (2) reported that intrathecal bolus infusion of chondroitinase ABC, which degrade chondroitin sulfate glycosaminoglycan chains, into crush lesions at C4 in the cervical dorsal column promoted regeneration of corticospinal tract axons and improved motor function. These reports suggest that degradation of CSPGs is a candidate of therapeutic strategies for SCI.

Materials and Methods
Using MASCIS impacter, rat SCI was produced by a 10 g weight dropping from 25mm height at eleventh thoracic vertebral level. Immediately after the SCI, SB203580 (10gμ), a selective p38 MAPK inhibitor, was injected intrathecal from 4-5th lumbar intravertebral space (SB group). For the vehicle rats, SB204274 (10gμ), a negative control of MAPK inhibitor, was injected (vehicle group).

The rat hind-limb motor function was evaluated by the conventional scoring system using BBB scale (Basso et al.) at 3 days, 1 and 2 weeks after the SCI.

To determine the amount of CSPGs and neurocan, western blot analysis was performed. Supernatant was taken from homogenized spinal cord tissue in 10mm length with PBS at 2 weeks after SCI. Supernatant was incubated with chondroitinase ABC. Sample solution was electrophoresed in 4% SDS-PAGE gel. Proteins were transferred to a PVDF membrane and incubated with anti-proteoglycan dDi-6s mAb (3B3) or anti-neurocan mAb (1G2), and following visualized with peroxidase-conjugated anti-mouse IgG and DAB substrate kit. For western blot analysis, the damaged spinal cord was harvested at 2 weeks after the SCI, and 10 μm axial frozen sections were prepared. Slices on the slides were exposed to anti-GFAP with anti- Chondroitin sulfate (CS56) or anti-neurocan (1G2) antibodies for double staining of astrocytes/CSPGs and astrocytes/neurocan. Then, slices were exposed to secondary antibody, Cy3-conjugated anti-rabbit IgG for astrocytes, and FITC-conjugated anti-mouse IgM for CSPGs and neurocan. The sections were observed under fluorescent microscope.

Results
BBB scores of both SB and vehicle group at 3 days after the SCI were 0 point, no observable hind-limb movement. At one week after the SCI, the average score of SB group was higher than that of vehicle group, but there was not significant difference between the groups. At 2 weeks after SCI, more improvement of hind-limb motor function was observed. BBB score in SB group (9.75 ± 1.18; n = 8) was significantly higher than that of vehicle group (5.73 ± 1.1; n = 8).

Using western blot analysis, CSPGs in the spinal cord at 2 weeks after the injury were detected by 3B3 antibody. The quantified CSPGs production after SCI was inhibited in SB group compared with that of vehicle group (64.0 ± 4.9; p = 0.04). Similarly, one of CSPGs, detected by 1G2 antibody was significantly inhibited in SB group compared with vehicle group (39.4 ± 4.0; p = 0.024).

Histologically, CSPGs was detected in damaged spinal cord around the cavity. In the vehicle group, CS56 positive cells and extracellular matrix were strongly stained at the edge of cavity and spread to remaining tissue. Most of CS56 positive cells were overlapped with GFAP-positive cells. On the other hand, CS56 positive cells and tissue were poorly stained in the SB group. Similarly, neurocan recognized by 1G2 antibody was detected around the cavity after SCI. In the vehicle group, neurocan was widely detected at remaining tissue around the cavity and overlapped with GFAP positive cells. However, dyeing affinity of 1G2 was small in area and poorly stained in the SB group.

Discussions
There are many reports that p38 MAPK signaling is related to cell apoptosis and inflammatory responses after central nervous system damage. Horiuchi et al. (3) reported that continuous intrathecal p38 MAPK inhibitor administration improved the motor function in a spinal cord compression-ischemia model in rats. We previously reported (ORS 2008) that p38 MAPK inhibitor reduced neurocan production from EGF-stimulated cultured reactive astrocyte in vitro. In present study, intrathecal administration of p38 MAPK inhibitor reduced CSPGs including neurocan production after SCI in vivo.

In recent studies, CSPGs produced in injured spinal cord are reported as inhibitory factor of neuronal regeneration. McKeon et al. (4) reported CSPGs induced neurite retraction and growth cone collapse in vitro. Davies et al. (5) reported that suppression of CSPG expression by decorin promoted axon growth after rat spinal cord injury. In this study, inhibition of CSPG production by p38 MAPK inhibitor improved rat hindlimb motor function after SCI.

References