SB203580, A SELECTIVE INHIBITOR OF P38 MITOGEN-ACTIVATED PROTEIN KINASE, IMPROVES HIND-LIMB MOTOR FUNCTION AFTER EXPERIMENTAL SPINAL CORD INJURY

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Introduction
By the recent progress of neuroscience, pathophysiological mechanisms of several neurodegenerative processes have been clarified and many potential candidates for therapeutic targets have emerged, i.e. neurotrophic factors, glutamate receptor antagonists, free-radical scavengers, etc. However, only a few methods including application of methylprednisolone have actually been applied for acute stage of traumatic spinal cord injury [1]. Therefore, novel therapeutic methods based on modern basic neuroscience should be developed for spinal cord injury. Activation of p38- mitogen-activated protein kinase (MAPK) has been reported to be one of the key mechanisms for apoptosis processes [3]. P38 MAPK has also reported to inhibit inflammatory responses in progression of neurodegenerative processes [2]. In the present study, inhibition of p38 MAPK by SB203580, a selective inhibitor of p38 MAPK, was tested against the neurological deficit following the experimental spinal cord injury in rats.

Materials and Methods
Spinal cord mild compression model
Wistar rats (250 g weight female obtained from Clea Japan, Tokyo, Japan) were used for this study. Under general anesthesia using halothane, the rat spinal cord was carefully exposed by removal of vertebral lamina at the level of 11th thoracic vertebra. The direct compression was performed using a 20 g weight. The part contacting the dura was made of soft silicone to prevent violent injury which would result in axonal tear or haemorrhage in the spinal cord. The weight was gently placed extradurally on the thoracic spinal cord for 40 minutes. The results were evaluated by comparison with sham animals, which underwent laminectomy without spinal cord compression. The research protocol was accepted by the ethical committee for animal experiments of Ehime University (Ehime, Japan).

Drug application
SB203580 (Calbiochem San Diego, CA, USA) was applied intrathecally using a micro-osmotic infusion pump (Alzet, Durect co., Cupertino, CA, USA). A thin polyethylene tube (0.28 mm in diameter; Becton Dickison) was inserted into the subarachnoid space at the 4th-5th lumbar intervertebral level just after the spinal cord injury. SB203580 was infused into the subarachnoid space for 1 week. The vehicle animal received saline infusion using a micro-osmotic infusion pump.

Analysis of the animal behavior
The animal behavior was analyzed using Scanet MV-10 (MATYS co. Tokyo, Japan). Infrared rays in the box detect animals passing through which are then recorded by the detector. Hind-limb function was assessed by counting the frequency of vertical movement using the detector (13 cm above the floor). Rats often assume a posture of lifting its fore-limbs and supporting their weight only on the hind limbs. In the present paper, we call this vertical movement ‘standing’. Rats usually take this standing posture about 100-120 times per hour. Rats often raised their head without lifting their fore-limbs. The detector was set high enough to prevent recognition of such small vertical movement. We theorize that decrease of the standing frequency reveals the severity of thoracic spinal cord injury. The measurement was performed for 1 hour in a dark, silent room.

Histological examination
Myelin structure was stained by Luxol fast blue. Apoptotic cells in the sections were detected by terminal deoxyribonucleotidyl transferase (TdT) -mediated dUTP-biotin nick end labelling (TUNEL) staining according to the assay protocol of the kit (Apoptosis in situ detection kit, 53501, Wako chemicals Co. Osaka, Japan). Photographs were taken and the number of TUNEL-positive cells was counted by three individuals who did not know any information about the pictures. The average number of labeled cells was obtained from the sections of six animals in each group and data was expressed as the number of cells per area of whole lateral funiculi.

Results
SB203580 was infused at the rate of 1 μg/day for one week. The motor function of hind limb function by measuring frequency of ‘standing’ was evaluated at one, two and three weeks after the spinal cord injury. The average of standing frequency in sham-operated rats was 117 ± 2 times/hr which was quite similar to that in normal animals without operation (115 ± 8 times/hr). One week after the injury, standing frequencies of both SB203580 perfused animals and vehicle animals decreased to about half of that in sham animals: 55 ± 17 times/hr and 65 ± 9 times/hr in SB203580 perfused animals and vehicle animals, respectively. At this time point, no significant effect of SB203580 was observed. However, at two weeks after the injury, frequency of standing of SB203580 perfused rats recovered to 104 ± 14 times/hr which is significantly (p < 0.05, ANOVA followed by Fisher’s PLSD) higher than that of vehicle animal (63 ± 7 times/hr). Three-weeks after the injury, standing frequency in SB203580 perfused animals maintained the recovered level (102 ± 16 times/hr). On the other hand, no recovery of standing frequency was observed in vehicle animals through the experimental period. Myelin structure was observed by Luxol fast blue staining. In sham animal spinal cord sections, white matter of spinal cord preserved its myelin structure. In our model was the thoracic spinal cord, descending tract in white matter, the lateral funiculi was most likely responsible for degeneration of hind limb function. From one to two weeks after the spinal cord injury, myelin degradation in lateral and dorsal funiculi progressed mainly in the central part of the spinal cord. The continuous intrathecal infusion of SB203580 almost completely preserved the myelin degradation in both lateral and dorsal funiculi at two weeks after the spinal cord injury. To evaluate apoptotic cell death in pyramidal tract, we counted the number of cells stained by the TUNEL technique in lateral funiculi. In vehicle animals, few TUNEL positive cells were observed at 3 days and 5 days after compression (7.22 ± 1.96, and 5.77 ± 2.35 cells/area respectively). The number of TUNEL positive cells increased to 77.88±11.04 and reached a peak at one week after compression. At two weeks after compression, the TUNEL positive cells were still present (34.44±6.29). When the rats were treated with SB203580, the number of TUNEL positive cells was significantly lower than that in vehicle animals (one week: 13.44 ± 3.03, and two weeks: 4.44 ± 1.68).

Discussion
In the present study, we demonstrated that local (intrathecal) application of p38-MAPK inhibitor significantly reduced the histological and behavioral damage after experimental spinal cord injury. In our model was the thoracic spinal cord, descending tract in white matter, the lateral funiculi was most likely responsible for degeneration of hind limb function. From one to two weeks after the spinal cord injury, myelin degradation in lateral and dorsal funiculi progressed mainly in the central part of the spinal cord. The continuous intrathecal infusion of SB203580 almost completely preserved the myelin degradation in this area, indicating that the working site of p38 MAPK in this experiment was oligodendrocytes in the compression area. However, no remarkable change in motor neurons in lumbar spinal cord were observed by the treatment of SB203580 (data not shown). The neurons in the thoracic spinal cord do not directly control hind limbs. Therefore, pyramidal tract in lateral funiculi or fasciculus proprius posterior in dorsal funiculi were more important than neurons in this part for hind limb motor function.

The results in the present study indicate that the application of p38-MAPK inhibitor prevents the delayed progressive degeneration of oligodendrocytes in the injured area, and if the damage to the cord is not complete, remarkable recovery of motor function can be expected for traumatic spinal cord injury.

References

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