REAL-TIME DIRECT MEASUREMENT OF SPINAL CORD BLOOD FLOW AT THE COMPRESSION PART: RELATIONSHIP BETWEEN BLOOD FLOW AND MOTOR DEFICIENCY IN THE SPINAL CORD INJURY

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Introduction
Measurement of spinal cord blood flow is important for understanding mechanisms of spinal cord injury. Until now, there have been several reports of spinal cord blood flow measurement. 1) hydrogen clearance method [7], 2) radioactive tracer microsphere technique [2], 3) Laser Doppler flowmetry technique [4], have been generally used. Using these techniques, spinal cord blood flow was measured at distal or proximal from the compression part [6]. Measurement of blood flow at the compression part after decompression was also measured [5]. However, real-time direct blood flow measurement method at the compression part has not been reported. In the present study, we developed a novel device for the measurement of rat spinal cord blood flow at the compression part, and investigated the relationship between spinal cord blood flow and hind-limb function.

Materials and methods
A novel device for the measurement of rat spinal cord blood flow
Our blood flow measurement system was a combination of non-contact type Laser Doppler system and spinal cord compression device (Paper, Morino et al.). The Laser Doppler blood flow system (Unique Medical co., LTD., Tokyo, Japan) can measure the blood flow within 1 cm from the tip of the probe. This Laser Doppler probe was set in the weight of spinal cord compression device. The tip of the probe, which is the contact part to dura, was covered with transparent soft silicone (3mm in diameter). The Laser beam passed through the silicone, and the blood flow was directly measured during the spinal cord compression (Fig. 1). During the experiment, the rat systemic blood pressure was monitored from the tail. No remarkable change was detected by spinal cord compression.

Fig. 1 Schematic drawing of real-time blood flow measurement system during compression

Animal model of spinal cord injury
Female Wistar rats (250g, obtained from Clea Japan, Tokyo, Japan) were used in this study. Under general anesthesia using halothane, the rat spinal cord was carefully exposed by removal of the vertebral lamina at the 11th vertebra. The compression device including Laser Doppler was gently attached on the surface of dura. This point was recognized as 100% of the blood flow. Then, blood flow was continuously measured. The compression weight increased 1g in every 1 minute. After the maximum compression (complete ischemia), the weight was reduced gradually. After decompression, blood flow was measured for another 30 minutes. In some animals, laminectomy was performed without spinal cord compression (sham group).

Evaluation of neurological recovery
Hind-limb function was evaluated using BBB scale [1] and standing frequency [3] at 3, 7, 14 days after spinal cord compression. Hind-limb function was assessed by counting the frequency of vertical movement using Scanet MV-10 (MATYS co., LTD., Tokyo, Japan).

Data Analysis
For statistical analysis of the data except BBB score, an analysis of variance (ANOVA), followed by the Fishier's PLSD, was used. For statistical analysis of BBB score, Mann-Whitney's U-test was used.

Results
Five gram weight compression reduced spinal cord blood flow from 100% to 42.9 ± 14.5%. Ten gram weight compression reduced blood flow to 18.8 ± 8.9%. Almost complete ischemia was achieved by 20g weight. Further weight increase to 30-50g did not change the blood flow in spinal cord (Fig. 2). Thus 20g weight compression was supposed to be enough for complete ischemia in rat spinal cord.

In the next experiment, maximum ischemic conditions by 20 g weight were maintained for 20 minutes (20g-20min group, n=14) and 40 minutes (20g-40min group, n=14). Blood flow after decompression in 20g-20min group recovered to 100%. On the other hand, reperfusion were incomplete in 20g-40min group (68-74%). There were significant differences between two groups at 10, 20, and 30 minutes after decompression (Fig. 3).

Since our spinal cord compression is not severe, no statistical significant change in BBB scale was observed between 20g-20min group and 20g-40min group. Counting the standing frequency is much more sensitive than BBB scale in such a mild injury [3]. There was no significant difference in standing frequency between 20g-20min group and sham group until 14 days after the compression. At 3 and 7 days after compression, standing frequency in 20g-40min group were significantly (p < 0.05) less than that in sham animals and 20g-20min group.

Discussion
Several spinal cord compression models were used in the experiment for spinal cord ischemia/injury. However there was no report describing complete ischemia production by minimum compression force. In the present study, we showed that 20 gram was enough for complete ischemia production. This information should be very important for the design of spinal cord compression model in rats.

In the relationship between ischemic period and motor deficiency, we could observe ischemia induced motor function loss in the hind limbs, while 20-minutes ischemia did not produce motor deficiency. In the present study, using novel device for blood flow measurement system, we could observe real-time blood flow change during compression period. We found that ischemic duration was very important factor for the recovery of motor functions after spinal cord injury.

References