ADAMTS-13 of the central nervous system is produced by glial cells and up-regulated after spinal cord injury

INTRODUCTION: ADAMTS-13 is a member of A Disintegrin And Metalloproteinase with Thrombospondin motifs family, primarily synthesized in hepatic stellate cells. However, the expression of ADAMTS-13 in the central nervous system is not known. In this study, we investigated the expression and proteolytic activity of ADAMTS-13 in rat spinal cord after injury.

METHODS: Animal subjects and surgery: Postnatal 9 weeks female Sprague-Dawley rats were the subjects of this study. All aspects of animal care and treatment were carried out according to the guidelines of the experimental animal care committee of Nagoya University, School of Medicine. A dorsal column spinal cord lesion was performed at the T10 level using HI impactor system. RT-PCR and Real time RT-PCR: The total RNA of the rat spinal cord tissue and cells were isolated using RNeasy (Lipid) tissue Mini Kit (QUIAGEN) according to the manufacturer’s instructions. The cDNA product was used for each PCR, according to the following protocol: 35 cycles of denaturing at 94°C for 30s, annealing at 58°C for 30s, and elongation at 72°C for 60s. Real-time PCR was performed using a Light Cycler 480 Real-Time System. Fluorescent assay: Tissue sample from rat spinal cord was diluted in 100μl of assay buffer. FRETS-VWF73 in the assay buffer was added to each well. Fluorescence was measured at 30°C in a POWERSCAN 4 equipped with a 360-nm excitation filter and a 460-nm emission filter. Fluorescence was measured after 60 min. Cell culture: Primary cultured cerebellar granule neurons (CGNs) from postnatal 8 days Sprague-Dawley rats were used. Primary cultures of cerebral cortical astrocytes were prepared from newborn Sprague-Dawley rats. Microglia-enriched cultures were obtained using the method of Giulian et al. Immunohistochemistry & Immunocytochemistry: Sections of rat spinal cord and cells were incubated with the primary antibodies at 100×dilution in a blocking solution overnight at 4°C. After rinsing in PBS, the sections were incubated with the secondary antibody for 60 min at room temperature. Subsequently, the sections were rinsed in PBS, mounted with FluorSave, and examined by an Olympus model BX41 microscope fitted with the appropriate filters. Statistical analysis: Statistical significance was determined using the Student’s t-test. Values of p < 0.05 were considered to indicate statistical significance.

RESULTS: We demonstrated that ADAMTS-13 mRNA and proteolytic activity in rat spinal cord were significantly increased after injury. We firstly report that glial cells (astrocyte and microglia), not neuron, secrete ADAMTS-13 in central nervous system. In addition, ADAMTS-13 antigen was detected in astrocyte and microglia.

CONCLUSION: In summary, our data demonstrated that ADAMTS-13 was expressed in central nervous system, especially in the glial cells. ADAMTS-13 mRNA and proteolytic activity during rat spinal cord injury were up-regulated. This phenomenon may play a role in modulating vasculatization after spinal cord injury, or increased ADAMTS-13 degrades other substrates other than von Willebrand factor. Finally, we believe that scientifically clarifying ADAMTS-13 expression mechanism after spinal cord injury leads to treatment for the spinal cord injury patients.

Figure 1. (A) ADAMTS-13 mRNA expression in the injured spinal cord of rats ADAMTS 13 mRNA was up-regulated in the injured spinal cord. n=3 (B) ADAMTS-13 activity in the injured spinal cord by fluorescent assay. The proteolytic activity of ADAMTS-13 in the injured spinal cord was significantly increased compared with sham-operated rats. P<0.05 n=4

Figure 2. ADAMTS-13 mRNA in rat brain cells (neuron, astrocyte, microglia). ADAMTS-13 mRNA was detected in astrocyte and microglia.

Figure 3. Immunocytochemical staining of ADAMTS-13 antigen in cultured rat glial cells. ADAMTS-13 antigen was expressed in astrocyte and microglia. GFAP (A), ADAMTS-13 (B, E), CD11b (D), GFAP-ADAMTS-13 merge (C), CD11b-ADAMTS-13 merge (F).

Figure 4. Immunohistochemical staining of ADAMTS-13 antigen in rat spinal cord 1 week after injury (A-I). ADAMTS-13 antigen was merged with astrocyte and microglia.

Reference

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