Hyaluronan Oligosaccharides Promote Functional Recovery after Spinal Cord Injury in Rats.

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INTRODUCTION:
The extracellular matrix (ECM) surrounding neurons in the central nervous system (CNS) forms a lace-like structure, the so-called perineuronal net (PNN). PNN is composed of hyaluronan (hyaluronic acid, HA), proteoglycans and tenacin R. Although PNN surrounding GABAergic interneurons is prominent, it can be detected on virtually all neurons. In contrast to extensive investigations of the effects of PNN-degrading enzymes on the structural rearrangement of neuronal networks, little attention has been paid to degradation products. In the present study, we investigated biological effects of HA oligosaccharides, preparing HA oligosaccharides composed of 2-12 saccharides (HA2-HA12), and applied them to spinal cord injury sites in a rat model.

METHODS:

Animal subjects and surgery: Postnatal 9 weeks female Sprague-Dawley rats were the subjects of this study. A dorsal column spinal cord lesion was performed at the T9 level and intrathecal continuous dosing of various HA oligosaccharides composed of 2-12 saccharides (HA2-HA12), High molecular weighted (HMW)-HA, and Saline with an osmotic pump was done. Evaluation of motor functional recovery was performed after the injury.

Cell culture: Primary cultured cerebellar granule neurons (CGNs) from postnatal 8 days Sprague-Dawley rats were used.

Neurite outgrowth assay: P8 rat CGNs were dissected, dissociated and plated at a density of 1 × 10^5 cells per ml on immobilized substrates (PLL, HA2-12, HMW-HA). Cells were cultured for 24h before fixation with 4% paraformaldehyde and staining with a neuron-specific anti-β-tubulin antibody. Neurite lengths were measured from at least 150 neurons per condition, from duplicate wells and from three independent experiments.

Assessment of neuroprotective activity: To assess the neuroprotective activity of HA oligosaccharides, we added various HA oligosaccharides preparations to neuronal cell cultures 24h before the administration of NMDA in various concentrations. Cells were cultured for an additional 24h, and the number of surviving neurons were counted with PI-calcein AM stain. In addition, neuronal cell death was estimated by measuring the activity of lactate dehydrogenase (LDH) released from damaged or destroyed cells into culture media.

Immunohistochemistry: Sagittal sections made from 2 weeks after the injury and treatment were set as 20-μm thickness. Then, incubated overnight at 4°C with polyclonal rabbit anti-ibα-1 antibody for microglia staining, and monoclonal mouse anti-CD68 antibody for activated microglia staining.

Anterograde labeling of cortico-spinal tract (CST): Eight weeks after injury, descending CST fibers were labeled with biotin-dextran amine (BDA). The degree of BDA uptake was assessed by counting the total number of fibers in the cross-section 10 mm rostral to the lesioned site, where the CST was intact. For quantification of the number of labeled corticospinal axons 10 mm caudal to the lesion site, labeled fibers were counted in the gray matter, the dorsal CST area (i.e., normal locations of the dorsal CST), or the white matter (excluding the dorsal CST area), and that number was divided by the number of labeled corticospinal axons 10 mm above the lesion.

RESULTS: Functional improvements in all groups showed significant differences (p<0.001, F value = 65.05: Repeated measure ANOVA). The difference between HA4 and Vehicle was significant (p=0.012: Posthoc Tukey’s test). However, neither the other HA oligosaccharides (i.e., HA6, 8, 10 and 12) nor the HMW-HA were associated with such an ameliorative effect (Fig. 1). There were significantly more BDA-positive fibers in the HA4-treated group in the region 10 mm caudal to the lesion, particularly in the gray matter of this region (Fig 2A). Thus, HA4 treatment contributed to axonal regeneration/sprouting after SCI. Iba1-positive cell accumulation in the lesion was significantly reduced in HA4-treated rats 2 weeks after injury, although there was no difference between HA4 treatment and vehicle control 1 week after injury (Fig. 2B). ED1-positive cell accumulation in the lesion was significantly reduced in HA4-treated rats both 1 and 2 weeks after SCI (Fig. 2C). HA4 was shown to exert inhibitory effects on microglial/macrophage recruitment, especially activated ED1 positive microglia which would play a crucial role in post traumatic inflammation in these lesions. Although, HA oligosaccharide showed no effect on neurite outgrowth (Fig 3A), neuroprotective effect of HA4 was significant (Fig 3B, C).

CONCLUSION: HA4 treatment enhances axonal regeneration/sprouting and suppresses accumulation of Iba-1-positive microglia and ED1 positive activated microglia.

SIGNIFICANCE: The present results therefore suggest the intriguing possibility that HA4 could be incorporated into a useful strategy for the treatment of neuronal injuries.

Figure 1. The result of BBB score. Rats with HA2 and 4 treatment showed preferable motor functional recovery. The difference was significant

Figure 2. HA4 treatment enhances axonal regeneration/sprouting and suppresses accumulation of Iba-1-positive microglia and ED1 positive activated microglia.

Figure 3. Effects of HA oligosaccharides on neurite outgrowth and neuroprotection. (A) Data represent average neurite length±SD. (B) Data represent the average ratio of PI-positive cells. *p=0.02 vs. 100 μM NMDA administration. (C) Lactate dehydrogenase (LDH) activity in culture media 24h after NMDA administration. Data represent the average optimal density value (550nm). *p=0.03 vs. 100 μM NMDA administration.