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Research article

A combination of keratan sulfate digestion and rehabilitation promotes anatomical plasticity after rat spinal cord injury

Yoshimoto Ishikawa [a,b], Shiro Imagama [a], Tomohiro Ohgomori [c], Naoki Ishiguro [a], Kenji Kadomatsu [b,*]

[a] Department of Biochemistry, Nagoya University Graduate School of Medicine, Showa-ku, Nagoya 466-8550, Japan
[b] Department of Orthopedics, Nagoya University Graduate School of Medicine, Showa-ku, Nagoya 466-8550, Japan
[c] Department of Developmental Molecular Anatomy, Graduate School of Medical Sciences, Kyushu University, Higashi-ku, Fukuoka 812-8582, Japan

HIGHLIGHTS

- KS-digestion plus rehabilitation was evaluated in a spinal cord injury model.
- KS-digestion/rehabilitation tended to improve functional plasticity.
- KS-digestion and rehabilitation synergistically improved anatomical plasticity.
- This effect was comparable with that of CS-digestion/rehabilitation.
- KS-digestion/rehabilitation might widen the therapeutic window of neuronal injury.

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ABSTRACT

Functional recovery after neuronal injuries relies on neuronal network reconstruction which involves many repair processes, such as sealing of injured axon ends, axon regeneration/sprouting, and construction and refinement of synaptic connections. Chondroitin sulfate (CS) is a major inhibitor of axon regeneration/sprouting. It has been reported that the combination of task-specific rehabilitation and CS-digestion is much more effective than either treatment alone with regard to the promotion of functional and anatomical plasticity for dexterity in acute and chronic spinal cord injury models. We previously reported that keratan sulfate (KS) is another inhibitor and has a potency equal to CS. Here, we compared the effects of KS- or CS-digestion plus rehabilitation on recovery from spinal cord injury. Keratanase II or chondroitinase ABC was locally administered at the lesion after spinal cord injury at C3/4. Task-specific rehabilitation training, i.e., a single pellet reaching task using a Whishaw apparatus, was done for 3 weeks before injury, and then again at 1–6 weeks after injury. The combination of KS-digestion and rehabilitation yielded a better rate of pellet removal than either KS-digestion alone or rehabilitation alone, although these differences were not statistically significant. The combination of CS-digestion and rehabilitation showed similar results. Strikingly, both KS-digestion/rehabilitation and CS-digestion/rehabilitation showed significant increases in neurite growth in vivo as estimated by 5-hydroxytryptamine and GAP43 staining. Thus, KS-digestion and rehabilitation exerted a synergistic effect on anatomical plasticity, and this effect was comparable with that of CS-digestion/rehabilitation. KS-digestion might widen the therapeutic window of spinal cord injury if combined with rehabilitation.

1. Introduction

Functional recovery after neuronal injuries relies on the reconstruction of neuronal networks. Maximization of these functional and anatomical plasticities is desirable as a therapy for various diseases, such as spinal cord injury (SCI) and stroke. However, it has been hard to achieve ideal reconstruction of neuronal networks, and no efficient therapeutics have been established for these diseases. On the other hand, it has become accepted that rehabilitation promotes functional recovery to some extent [1,2,6], probably by promoting the reconstruction and refinement of specific networks.

Reconstruction of neuronal networks may involve several processes, i.e., sealing of injured axon ends, axon regeneration/

* Corresponding author at: Department of Biochemistry, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan. Tel.: +81 52 744 2059; fax: +81 52 744 2060.
E-mail address: kkadoma@med.nagoya-u.ac.jp (K. Kadomatsu).

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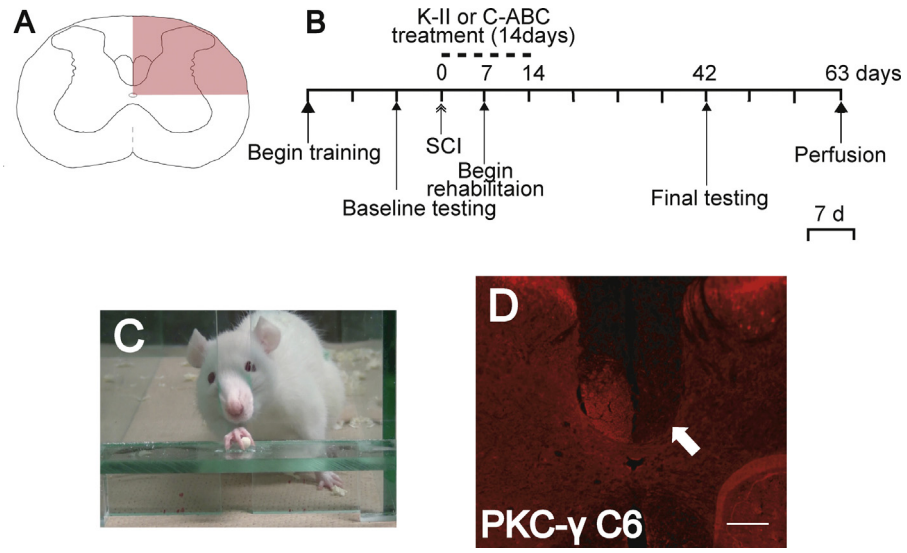

Fig. 1. In vivo experimental design.
(A). In schematic cross section of the spinal cord. The red area indicates the unilateral dorsolateral lesion at level C3/4, which includes the unilateral dorsal CST. (B). Flow of the experiments. (C). The single pellet reaching training required forearm training. (D). PKC-γ staining was performed to assess the CST lesion. At the C6 transverse section (caudal to the lesion), PKC-γ could not be detected on the lesion side. Scale bar, 200 μm.

sprouting, and construction and refinement of synaptic connections. Therefore, a comprehensive understanding of these processes is required to establish therapies for neuronal injuries [9]. Axons of the adult mammalian central nervous system (CNS) do not regenerate or sprout after injuries due to the low intrinsic regeneration capacity and emerging inhibitory molecules. Chondroitin sulfate (CS) is a strong inhibitor for axon regeneration/sprouting [13]. Ablation of CS by its degrading enzyme chondroitinase ABC (C-ABC) promotes not only axon regeneration/sprouting but also functional recovery [3,12]. Furthermore, CS-digestion/task-specific rehabilitation improved dexterity recovery in both acute and chronic SCI [4,16].

CS belongs to a class of long sugar chains known as glycosaminoglycans, which are composed of repeating disaccharide units. Along with CS, keratan sulfate (KS), heparan sulfate, and hyaluronan belong to the glycosaminoglycans. We previously reported that KS acts as an inhibitor for axon regeneration/sprouting [7,8]. 5D4-reactive KS-deficient mice (GlcNAc6ST-1 knockout) showed enhanced axon regeneration/sprouting and better motor function recovery as compared with wild-type mice [8]. Local administration of keratanase II (K-II), a KS-specific degradative enzyme, can also ameliorate SCI [7]. Notably, the effect of KS-digestion is comparable to that of CS-digestion [7].

In this study, we compared the effects of KS- or CS-digestion plus rehabilitation on recovery from spinal cord injury.

2. Material and methods

2.1. Animal and experimental groups

Adult female Sprague–Dawley rats weighing 200–230 g were used in this SCI study. All the animals ($N = 58$) received the SCI at C3/4, and K-II, C-ABC or saline with or without training in the single pellet reaching task. The animals were divided into six experimental groups: a K-II training group ($N = 5$); K-II no-training group ($N = 5$), C-ABC training group ($N = 5$), C-ABC no-training group ($N = 5$), saline training group ($N = 5$), and saline no-training group ($N = 5$). For histological analysis of the association of PNNs and KS, un-injured normal rats ($N = 3$) were used.

2.2. Surgical procedure

The animals were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). After C4 laminectomy, we exposed the dura mater and induced a dorsolateral cut injury between C3 and C4 ipsilateral to the preferred paw (Fig. 1A). A cut was made with the tips of sharpened fine micro-blade inserted 2 mm in depth into the spinal cord. This injury included the descending dorsal CST and the ascending sensory dorsal columns. Immediately, after the dorsolateral cut injury, we performed C6 partial laminectomy and inserted a thin silicone tube with an osmotic mini-pump into the subarachnoid cavity, and set the tube tip at the C3 level under a surgical microscope. This tube was sufficiently soft and thin that we could minimize damage to the spinal cord. The osmotic mini-pumps (Model 2006; ALZET, Cupertino, CA; 200 μl of solution, 0.5 μl/h, 14 d delivery) were filled with K-II (0.05 U/200 μl; Seikagaku), C-ABC (0.05 U/200 μl; Seikagaku), or saline (as a vehicle control). The tube was sutured to the spinous process to anchor it in place, and the mini-pump was placed under the skin on the animal's back. Afterward, the muscles and skin were closed in layers [7,14]. The dose of C-ABC or K-II was decided from a previous report [7].

One day following SCI, rats were visually inspected and evaluated in regard to the degree of paralysis. Rats with bilateral deficits or deficits of the shoulder were excluded from the study in all groups as they were inappropriate for further evaluation [5]. All animals were treated and cared for in accordance with the Nagoya University School of Medicine Guidelines pertaining to the treatment of experimental animals.

2.3. Single pellet reaching task

Before SCI, rats were trained to reach through a slot (1.5 cm wide) in an acrylic box (15 cm × 36 cm × 30 cm) to grasp food pellets of 45 mg each (Bio-serv, USA). The pellets were set in a small indentation on a tray and offered one at a time (the pellets were 2 cm away from the front wall at a height of 3 cm above the elevated grid floor) [11]. Success rates per session were calculated as the number of pellets successfully grasped and eaten out of 20 pellets offered. Starting on day 7 after SCI, training was performed for 20 min per day, 5 days per week for 5 weeks (Fig. 1B and C).