The effects of combining chondroitinase ABC and NEP1-40 to the corticospinal axon growth in organotypic co-cultures of brain cortex and spinal cord

Toshio Nakamae, Nobuhiro Tanaka, Kazuyoshi Nakanishi, Naosuke Kamei, Hirofumi Sasaki, Takahiko Hamsasaki, Kiyotaka Yamada, Risako Yamamoto, Yu Mochizuki, Mitsuo Ochi
Department of Orthopaedic Surgery, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan
toshinakamae@hiroshima-u.ac.jp

Introduction: Recent studies have demonstrated functional and histological recovery of injured spinal cord in animal models. However, there are non-permissive milieu surrounding the injured spinal cord due to the inhibitory factors, especially chondroitin sulphate proteoglycan (CSPG) and Nogo. CSPG is originally the extracellular matrix contained within a glia scar. Nogo regulates the abnormal axon growth in the embryonic stage. When the spinal cord was injured in adult mammals, CSPG and Nogo inhibit the axon growth. It is necessary to control these inhibitory factors in the axon regeneration strategy. Recent studies have reported that digestion and blocking these factors induce promoting the axon growth and functional recovery. For example, chondroitinase ABC (ChABC) induces axonal regeneration and functional recovery by degrading chondroitin sulphate (CS)-GAG and Nogo-66/1-40 antagonist peptide (NEP1-40) promotes axon growth after SCI due to blocking Nogo-66/NgR interaction. But it is not enough to assess the effects of axon growth quantitatively. Furthermore, there is no study that addresses the effect of combining ChABC and NEP1-40 in the spinal cord injury.

Oishi and his colleagues suggested an organotypic co-culture system using brain cortex and spinal cord from neonatal rats [1]. The advantage of this co-culture system is to assess corticospinal tract (CST) axon growth quantitatively and facilitate the analysis of factors that regulate axonal growth [2]. The purpose of this study was to examine whether the combination of these two extrinsic approaches, ChABC and NEP1-40 infusion, facilitate axonal growth from the brain cortex to the spinal cord in organotypic co-culture.

Materials and Methods: Organotypic co-culture of brain cortex and spinal cord were prepared as reported previously. Brains and thoracic spinal cords were collected from SD rats on postnatal day 7. The brains were sectioned using a Vibratome. The region of the sensorimotor cortex was dissected from the coronal sections, and thoracic spinal cord was dissected in the sagittal plane. The dissected cortex and spinal cord were placed on membranes, in the 1ml of serum-based medium. The cortex and the spinal cord were incubated for 1 day, then, on the second day, the spinal cord pieces were aligned next to the white matter of the cortex. The co-cultures were incubated in a humidified atmosphere with 5% CO2 at 37°C. The medium was replaced every 3 days. The co-cultures were incubated for up to 14 days.

Drugs were infused into the co-culture just after the cortical tissue and the spinal cord tissue contacted one another. They were dropped on the junction of brain cortex and spinal cord in the quantity of 2μl every 3 days during incubation. To degrade CSPG, ChABC was delivered to the co-culture in the concentration of 1U/ml (ChABC group). To block Nogo-66/NgR interaction, NEP1-40 was applied in the concentration of 0.4μg/ml (NEP group). To examine the effects of combination, these two drugs were infused together to the co-cultures (combined group). For the control, infusion of only medium was performed (control group).

Axon projections from the cortex to the spinal cord were labeled with DiI (Fig.1). The co-cultures were fixed for 5 days in 4% paraformaldehyde at 4°C. Small crystals of DiI were placed on the center of the cortex, and the co-cultures were incubated for another 14 days in 0.1M phosphate buffer. We counted the number of labeled axon passing through a reference line running parallel to the junction between the brain cortex and spinal cord 500, 1000 and 1500μm from the junction.

Results: In the control group, the average number of axons that extended 50μm past the junction was 0.6±0.3 (n=12 cultures/group). While, in the ChABC group and in the NEP group, the average number of axons that extended 50μm past the junction was 3.9±0.4 (n=12 cultures/group) and 5.1±1.3 (n=8 cultures/group), respectively. The average number of axons in the ChABC group and in the NEP group was significantly greater than that in the control group (P<0.05). In the combined group, the average number of axons that extended 50μm past the junction was 4.9±1.0 (n=12 cultures/group). This was significantly greater than that in the control group, but this was not significantly greater than that in the ChABC group or in the NEP group (Fig.2).

Discussion: In this study, axon growth was enhanced by infusing of ChABC or NEP1-40 compared with the control group. ChABC and NEP1-40 have the action that promotes corticospinal axon growth. On the other hand, combination of ChABC and NEP1-40 did not promote corticospinal axon growth in organotypic co-culture system in comparison to the infusion of the ChABC or NEP1-40 solo. In this co-culture system, ChABC or NEP1-40 single-infusion had effects equivalent to these combining infusion in axon growth. By facilitating the analysis of factors around these organotypic co-cultures, there is a possibility to elucidate the intracellular pathways that ChABC and NEP1-40 affect. In this study, these results provide insight into the development of new assays and strategies to enhance axon regeneration in injured spinal cord.