Neural Regeneration in Spinal Cord Injury using Combination of Photoreactive Gelatin and Fusion Protein of Hepatocyte Growth Factor

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Disclosures:

Introduction: A combinatorial approach involving scaffolds, neurotrophic growth factors, and cells is believed to be the ideal therapy for effectively repairing spinal cord injury (SCI). However, methods to deliver growth factors and cells to injured sites efficiently have not yet been well established. Hepatocyte growth factor (HGF) is one of the neurotrophic growth factors to neural regeneration. We created a fusion protein of collagen-binding HGF (CBD-HGF), which has high collagen binding affinity and high activity in the bound state of collagen. Hydrogels have been studied as one of biomaterials for spinal cord injury. In the present study, we used photoreactive gelatin (PG) as a scaffold, which is a kind of hydrogel and can be solidified by visible light. This gelatin also can encapsulate growth factors and cells. We examined whether the combinational methods of PG and CBD-HGF can be effective for the spinal transected mouse model.

Methods: In vitro experiment:
Collagen-binding assay of CBD-HGF and native HGF from PG:
For investigating the binding activity of CBD-HGF and native HGF for PG, a total of 30ul 15% gelatin with 0.05% RB containing growth factor (CBD-HGF 100ng or native HGF 100ng) was applied in 24 wells plate and gelled by irradiation with visible light for 2 minutes. The sample was incubated with PBS 500ul at 37°C, and the gelatin containing the remained growth factor was collected at specific times (days 1, 3, and 7). After collection, the gelatins were mechanically disrupted by adding 500ul PBS. Concentration of HGF was measured by the enzyme-linked immunosorbent assay (ELISA) kits.

In vivo experiment:
Adult female C57BL/6 mice (8-9 weeks old) were anesthetized. A complete laminectomy was performed at T9 level. The injury was done as a complete transection at T9 including the dura mater and the whole circumference of the cord. All animals were divided into four groups receiving different treatments: (1) the group of control: no additional treatment; (2) the group of PG: 15ul of gelatin was implanted on transection site and gelled by irradiation with visible light for 1 minute; (3) the group of PG containing HGF: 15ul of gelatin dissolved 1ug HGF was implanted on transection site and gelled by irradiation with visible light; (4) the group of PG containing CBD-HGF: 15ul of gelatin dissolved 1ug CBD-HGF was implanted on transection site and gelled by irradiation with visible light. The Okayama University Animal Care and Use committee approved all animal experiments conducted in this study.

Motor function recovery comparisons among all groups were performed using the Basso Mouse Scale (BMS), analyzing hindlimb functional recovery after SCI. Animals were observed at seven days post injury and thereafter followed weekly for a total of 8 weeks post-injury. Electrophysiological experiments were performed at 8 weeks after injury. Motor-evoked potential (MEP) were elicited with stimulation of the spinal cord at the occipito-cervical interspace. Recording electrodes in the hindlimb were placed on the muscle belly (recording) and the distal tendon of the muscle (reference). Stimulus intensity was adjusted to 10-20% above the voltage at which the maximum amplitude of the initial peak of the evoked response was observed. The amplitude (μV) was measured from the initiation point of the first wave to its highest point. Immediately after MEP experiment, animals were transcardially perfused with 4% PFA and the spines were removed. Immunochistochemistry was performed on the following primary antibodies: anti-GAP43, anti-GFAP, and anti-MBP. The areas of tissue immunopositive for GAP43, GFAP, and MBP were quantified. All the data were expressed as mean ± SEM. P-values of less than 0.05 were considered to be significantly different.

Results: 1) Higher binding activity of CBD-HGF for PG
in vitro:
The value of CBD-HGF remained in PG at each time (days 1, 3, and 7) was significantly higher than that of native HGF (Fig. 1). This result demonstrated that CBD-HGF could strongly bind to PG by the effect of CBD.

2) Functional analysis in vivo:
We assessed locomotor functional recovery using the BMS scoring of open field walking (Fig. 2). Immediately after SCI, the animals exhibited complete hind limb paralysis (BMS score 0). Mice in PG containing CBD-HGF group had significantly higher BMS scores than other three groups on day 7 and thereafter.

3) Recovery of electrophysiology:
MEP amplitude of the PG containing CBD-HGF group was significantly larger than those of other groups (Fig. 3).
4) Immunohistochemistry
The immunopositive areas of GAP43 and MBP in the group of PG containing CBD-HGF were significantly larger than those of other groups.

**Discussion:** In the present study, the combination therapy was applied to mouse transection model. In hind limb motor functional analysis using BMS, groups treated with PG and CBD-HGF had significantly higher scores than the others at all times for 7 days post injury. Even in MEPs and immunohistochemistry, groups treated with gelatin and CBD-HGF showed significantly better results than the others. This study suggests that pleiotropic functions of HGF in the nervous system work effectively on the transection site with the combination of PG and CBD-HGF. A report showed that required HGF levels are several times higher than the control level when therapeutic effects of HGF were expected in the injured spinal cord. The characteristic of this gelatin that can keep growth factors could bring therapeutic effects. The amount of HGF and CBD-HGF used in this study was smaller compared to the previous report. We consider that a combination of PG and CBD-HGF enable therapeutic effects with low dose administration.

**Significance:** The combinational therapy of PG and CBD-HGF showed positive therapeutic effects on mouse spinal cord transection model. PG could provide the optimum environment for neural regeneration and synergistic effect with CBD-HGF. This method can be a promising vehicle for severe spinal cord injury such as transection.

**Acknowledgments:**

**References:**
Fig. 1.
Amplitude

Fig. 3. * $p<0.001$ ** $p<0.01$
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