Anti-Inflammatory Effect of Hepatocyte Growth Factor in Acute Phase of Spinal Cord Injury

Kentaro Yamane, Yohei Kagawa, Kensuke Shinohara, Noriyuki Watanabe, Aki Yoshida, Akihiro Matsukawa, Toshifumi Ozaki.
Okayama University Graduate School, Okayama, Japan.

Disclosures: **K. Yamane:** None. **Y. Kagawa:** None. **K. Shinohara:** None. **N. Watanabe:** None. **A. Yoshida:** None. **A. Matsukawa:** None. **T. Ozaki:** None.

Introduction: Inflammation that occurs after a spinal cord injury (SCI) causes secondary damage that worsenshe patient's prognosis. To lessen the damage and improve function, it is extremely important to control the inflammatory response at the site of injury. Hepatocyte growth factor (HGF) is a neurotrophic growth factor which also has a pleiotropic effect on the central nervous system. The reported therapeutic mechanisms of HGF in SCI include an anti-apoptosis effect on neurons and oligodendrocites, decreased grial scar formation, and promotion of neurogenesis and oligogenesis for neural stem cells. At the same time, it is reported in basic research that HGF has an anti-inflammatory effect by acting on immune cells, essentially by inhibiting macrophages from producing pro inflammatory cytokines and chemokines. However, it is unclear whether the anti-inflammatory properties of HGF function in the acute phase of SCI. We focused on the anti-inflammatory properties of HGF and examined whether HGF can modify the inflammatory response in the acute phase of SCI using mice compression models.

Methods: In vitro experiment

Gene transcription of bone marrow derived macrophages (BMDMs) under HGF exposure

To confirm whether HGF influences the gene transcription of M1 macrophages, we used lipopolysaccharide (LPS) stimulated BMDMs. BMDMs were incubated with or without HGF (50 ng/mL) and stimulated with 100 ng/mL LPS for 6 hours. Next, RNA was isolated from the BMDMs and reverse-transcribed. We used the following primers from Applied Biosystems: IL1b, IL6, TNFa, CXCL1, CXCL10, and CCL2. Quantitative real-time PCR was performed with mouse glyceraldehyde-3-phosphate dehydrogenase as a housekeeping gene on a TaqMan 7500 sequence detection system (Applied Biosystems), and fold expression was normalized using the ΔΔCt method.

In vivo experiment

We used a fusion protein of HGF with a collagen-binding domain (CBD-HGF) in addition to native HGF. CBD-HGF has a high collagen-binding affinity with increased activity in the bound state with collagen. The effect from a single dose administration will last at least for several days. Adult female C57BL/6 mice (8-9 weeks old) were anesthetized to perform a complete laminectomy at T9 level. We performed an extradural temporary closure of a vascular clip with 10g force around the exposed spinal cord for 1 min to cause an acute compression injury. We then divided the animals into 3 groups to receive 2 ul intrathecal injections of their specific treatment into the injured site: The control group received PBS, the native HGF group received 5.0×10-6 mol/l native HGF, and the CBD-HGF group received 5.0×10-6 mol/l CBD-HGF. The Okayama University Animal Care and Use committee approved all animal experiments conducted in this study. We examined gene transcription in the spinal cord at 24 hours and 72 hours after the SCI in the same manner described for the in vitro experiment. Motor function recovery comparisons among all groups were performed using the Basso Mouse Scale (BMS). Motor-

evoked potentials (MEP) were elicited at 6 weeks post injury. We used immunohistochemistry to determine infiltration of inflammatory cells. In samples collected on day 3 or day 7, we used anti-CD68, anti-Ly6g, and anti-CD3 antibodies. For samples collected 6 weeks post SCI, we used anti-GAP43, anti-GFAP, and anti-MBP antibodies. The areas of tissue immunopositivity were quantified with all data expressed as mean ± SEM. P-values of less than 0.05 were considered significantly different.

Results: 1) HGF decreased gene transcription of LPS-stimulated BMDMs

HGF significantly suppressed gene transcription of IL1b, IL6, CXCL10, and CCL2, which are pro-inflammatory cytokines and chemokines known to direct cell infiltration and proliferation in LPS-stimulated BMDMs after 6 hours. HGF also tended to decrease gene transcription of TNFa and CXCL1.

2) CBD-HGF decreased gene transcription of pro-inflammatory cytokines and chemokines after SCI

With IL6 and TNFa, the CBD-HGF group had a significantly lower level of gene transcription than the other two groups at 24 hours post SCI. With IL1b, CXCL10 and CCL2, the CBD-HGF group had a significantly lower level of gene transcription than the control group at 24 hours post SCI. With CXCL1, the CBD-HGF group tended to have less gene transcription than the other two groups at 24 hours post SCI. (Fig.1) The CBD-HGF group at 72 hours post SCI also tended to have less gene transcription compared to the other two groups. CBD-HGF's sustainable effect had a stronger influence on gene transcription than HGF's temporal effect.

3) Quantitative assessment of inflammatory cells in acute phase of SCI

The CBD-HGF group had a significantly smaller number of CD68 immunopositive cells compared to the control group on both day 3 and day 7 post SCI. (Fig. 2) The number of Ly6G immunopositive cells on day 3 in the CBD-HGF group was significantly smaller than that of the control group. T cell infiltration as measured by CD3 immunopositive areas was barely detectable on days 3 and 7 among all groups.

4) Functional analysis in vivo (Fig. 3)

The CBD-HGF group had significantly higher BMS scores than the control group starting on day 3, and the CBD-HGF BMS scores were significantly higher than the HGF group starting on day 7. There was no significant difference in the BMS scores between the control and HGF groups at any time post SCI.

5) Recovery of electrophysiology MEP amplitude of the CBD-HGF group was significantly higher than the amplitudes of the other groups. There was no significant difference in MEP amplitude between the control and HGF groups.

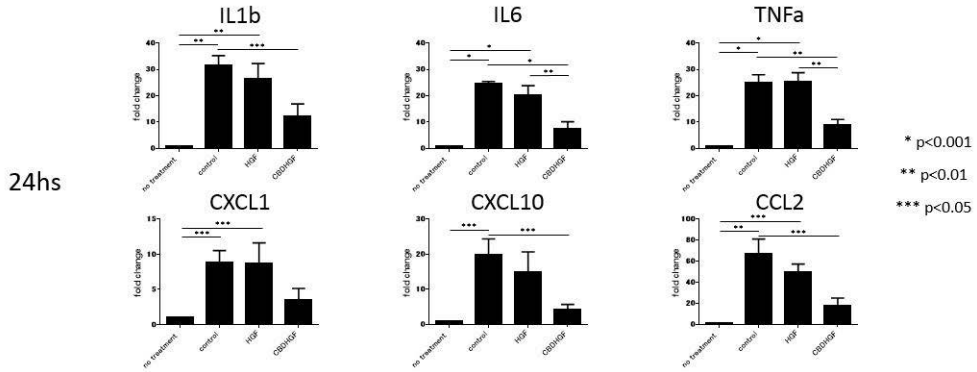
6) Immunohistochemistry of spinal cord 6 weeks post SCI

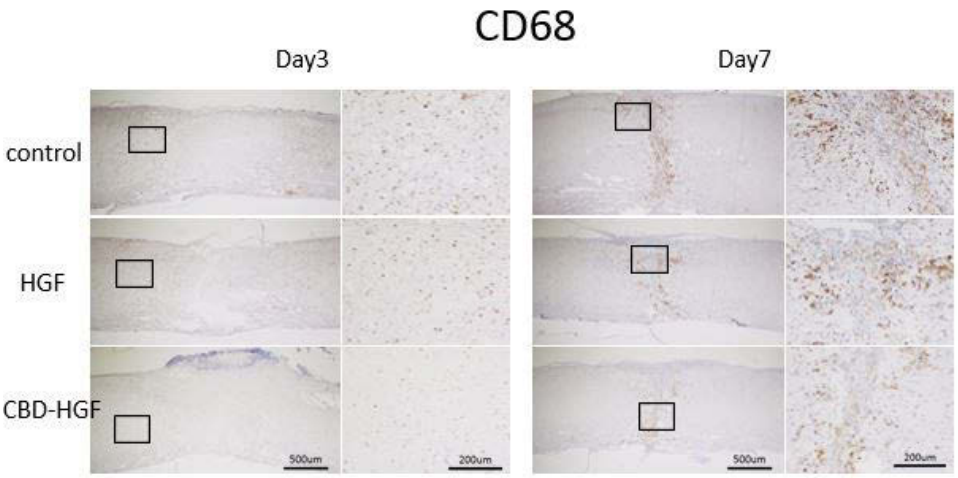
The immunopositive areas of GAP43 and MBP in the CBD-HGF group were significantly larger than the immunopositive areas of the other groups. On the other hand, the immunopositive areas of GFAP were significantly smaller in the CBD-HGF group than in the other groups.

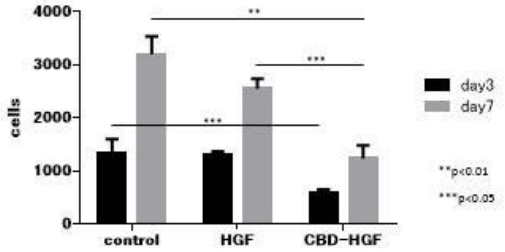
Discussion: In addition to the previously reported positive effects of HGF on a SCI, this study showed an important anti-inflammatory effect of HGF in the acute phase of SCI. Our results demonstrated that HGF administration immediately after a SCI first inactivated M1 microglia to reduce pro-inflammatory cytokines and chemokines. Next, it decreased infiltration of hematogenous macrophages and neutrophils into the injured site. Consequently, the whole inflammatory process was suppressed. In addition to the anti-apoptotic effect of HGF, its anti-inflammatory effect can help decrease secondary damage at the site of injury. We demonstrated this with the superior functional recovery (BMS score) starting on day 3 and the signal conductional recovery (MEP) of the CBD-HGF group. Even the immunohistochemistry results of GAP43, 5-HT, MBP, and GFAP show that inhibiting the inflammatory

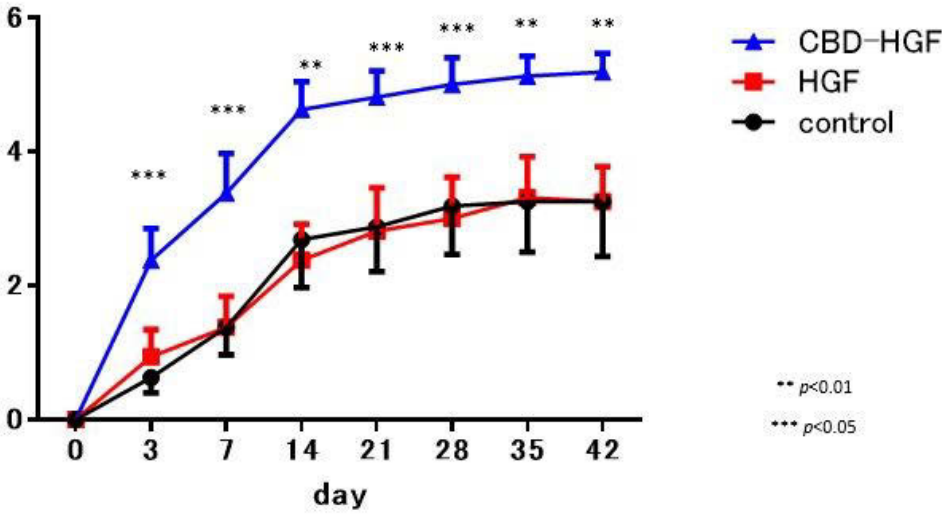
response in the acute phase of SCI can have a positive impact on neural regeneration, cell survival and even prevention of grial scar formation.

Significance: The anti-inflammatory effect of HGF has therapeutic value in the acute phase after a SCI by inhibiting macrophages/microglia. Our results show that the CBD-HGF's sustainable effect was of greater benefit in the inhibition of pro-inflammatory cytokines and chemokines than the temporal effect of native HGF.









ORS 2015 Annual Meeting

Paper No: 0128