Hepatocyte Growth Factor Promotes Endogenous Repair and Functional Recovery after Spinal Cord Injury; Preclinical Trial Using Recombinant Human HGF from Rodents to Primates

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Introduction: Hepatocyte growth factor (HGF) has been reported to promote neuronal survival and functional recovery in rodent models of cerebral ischemia [1] and amyotrophic lateral sclerosis [2]. The purpose of this study is to determine the therapeutic effects of HGF on spinal cord injury (SCI) in adult rats and establish a novel strategy using recombinant human HGF (rhHGF) in a cervical SCI model of common marmosets as a preclinical trial.

Materials and Methods: 1) To examine changes in the endogenous expression of HGF and its receptor c-Met after SCI, SCI was induced in adult female SD rats and the spinal cords were analyzed by real time PCR, ELISA and immunostaining at multiple time points after SCI. 2) To determine the therapeutic effects of HGF on injured spinal cord, herpes simplex virus (HSV) type-1 vectors containing rat HGF (HSV-HGF group) or LacZ (HSV-LacZ group) were injected into the spinal cord at the Th 10 level and SCI was induced 3 days later at the same level using an IH impactor. 3) Next, aiming at clinical application, contusive SCI was induced at Th 10 level in adult rats using IH impactor and at C5 level in common marmosets with modified MASCIS protocol [3], and rhHGF (rhHGF group) or PBS (PBS group) was administered intrathecally through the osmotic mini-pump for 4 weeks after the injury. 4) The injured spinal cords from each group were analyzed by immunoblotting and immunohistochemical staining. Functional recovery was evaluated by BBB scale for 6 weeks after SCI in rats and by our original scale for 12 weeks in common marmosets.

Results: 1) The HGF mRNA expression levels in the spinal cord gradually increased, peaking at 2 weeks after SCI, whereas the c-Met mRNA expression levels increased markedly within 1 day of SCI, suggesting that endogenous upregulation of HGF was insufficient to that of c-Met especially during the acute phase of SCI. HGF protein levels in the spinal cord also unchanged during the acute phase and gradually increased peaking around 4 weeks after SCI. In addition, no increase in the plasma HGF levels was found after SCI. In the intact spinal cord, c-Met-Immunoreactivity (IR) was observed in neurons and oligodendrocytes, but not in astrocytes. At 1 week after SCI, c-Met-IR was obviously observed in reactive astrocytes as well as in neurons and oligodendrocytes. 2) At 6 weeks after SCI, H.E. and luxol fast blue staining showed remarkable reduction in the damaged area and immunostaining using anti-ChAT antibody showed significantly larger number of survived motoneurons in the HSV-HGF group compared with HSV-LacZ group. Induction of caspase3 in both neurons and oligodendrocytes was significantly attenuated during the acute phase of SCI (Fig. 1A) and the number of RECA-1-positive newly formed blood vessels was significantly larger at 1 week after SCI in the HSV-HGF group. Moreover, regrowth of SHT-positive serotonergic fibers was promoted (Fig. 1B), which resulted in significantly better functional recovery after SCI in the HSV-HGF group. 3) Intrathecal administration of rhHGF, like HGF introduction by HSV-HGF vector, significantly reduced damaged area and promoted functional recovery in both rats (Fig. 2) and common marmosets, confirming the efficacy of rhHGF for SCI in rodents and primates.

Fig.2 Demyelination (LFB staining) and cavity formation (H.E. staining) were remarkably reduced in rhHGF group compared with those in PBS group. BBB scores after SCI also showed a significant improvement in hindlimb motor function in rhHGF group as compared with those in PBS group.

Discussion: We demonstrated for the first time that the injured spinal cord cannot produce a sufficient amount of HGF by itself, compared with the remarkable increase in c-Met expression especially during the acute phase of SCI, nor can HGF be supplied through an endocrine mechanism via blood supply. Introduction of exogenous HGF into spinal cords by HSV-HGF injection compensated for the deficiency of endogenous HGF upregulation and showed not only neuroprotective effects but also promoted angiogenesis and axonal regrowth after SCI. Furthermore, intrathecal administration of rhHGF also showed positive effects on histological changes and the motofunction after SCI even in common marmosets. This study demonstrates the efficacy of HGF for SCI and its possibility of clinical application.


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