Enoxaparin Promotes Recovery after Spinal Cord Injury

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INTRODUCTION: Receptor type protein tyrosine phosphatase σ (PTPRσ) regulates axonal regeneration/sprouting and its inhibition as a molecular switch through its clustering in response to glycan ligands. Cell surface heparan sulfate oligomerlizes PTPRσ and inactivate its enzymatic activity, leading to axonal growth promotion. Contrary, matrix-associated chondroitin sulfate monomerlizes PTPRσ and activates it. That leads to dephosphorylation of its specific substrates such as cortactin, resulting in axonal regeneration failure after injury. However, this molecular switch model has never been challenged or utilized in clinical situation. Here, we investigated the effect of Enoxaparin, worldwide approved anticoagulant consisting of heparin (an over-sulfated type of Heparan Sulfate) oligosaccharides with an average 45 kDa of molecular weight to axonal injury.

METHODS: We first examined whether these low molecular weight heparins were able to compete with chondroitin sulfate for PTPRσ. To do this, we carried out the competition assay on surface plasmon resonance (SPR). To further address the molecular interaction between these heparin oligosaccharides and PTPRσ, isothermal calorimetry (ITC) was used. To address whether these phenomena could occur on the surface on living cells, we employed in vitro phosphatase assay. We next made rat severe spinal cord injured models which were inflicted contusion injuries on the thoracic level (Th9) using a force of 200 kdyn to evaluate the effect of enoxaparin to spinal cord injury. These models were treated with subcutaneous saline and enoxaparin every day from 1day after spinal cord injury. We examined the motor function by BBB score, the sensory function by touch test and electrophysiological function by motor-evoked potential (MEP) monitoring. In addition, we performed histological analysis of the serial section of the spinal cord using sagittal sections and strained for GAP-43 and 5-HT.

RESULTS: SPR results showed that bound PTPRσ to chondroitin sulfate was rapidly eluted after injection of Enoxaparin (Figure1a), suggesting that these compounds were good competitors to occupy PTPRσ from chondroitin sulfate. ITC results showed that Enoxaparin and PTPRσ produced heat of reaction with approximately 1:6.5 binding stoichiometry (Figure1b), so we concluded that Enoxaparin can strongly interact with and induce clustering of PTPRσ. Overexpression of v-src, a proto-oncogene tyrosine kinase, greatly induced bulk protein tyrosine phosphorylation in cells, which was demonstrated by 4G10 antibody. Coexpression of PTPRσ diminished the immunoreactivity of 4G10. Treatment of cells expressing PTPRσ with Enoxaparin canceled the dephosphorylation by PTPRσ (Figure1c). The BBB score was significantly higher in Enoxaparin group than in the saline-administered control from 21st day after SCI (Figure2a). These results suggest that enoxaparin promotes motor functional recovery after spinal cord injury. The recovery of sensory function was significantly better in Enoxaparin group than in the saline-administered control from 42st day after SCI (Figure2b). At 84 days after injury, we performed motor-evoked potential (MEP) monitoring, in which an electric stimulus is given at the thoracic level (Th7), and a response is taken at the gastrocnemius muscles. The latencies and the durations of the MEP waves were measured. The latencies and the durations of enoxaparin group were shorter than that of saline. We performed histological analysis of SD rat spinal cords treated with enoxaparin or saline at 84 days after SCI. We counted the number of GAP-43 and 5-HT positive fibers 3mm rostral and 3mm caudal from the lesion site. The GAP-43 positive fibers was significantly higher in both the rostral and caudal regions in enoxaparin group than in saline group. The 5-HT positive fibers was significantly higher in the rostral regions in enoxaparin group than in saline group. On the other hand, there was no significant difference of the 5-HT positive fibers in the caudal regions between enoxaparin group and saline group.

DISCUSSION AND CONCLUSION: Our recent work showed that Heparan Sulfate (HS), which contained a lot of receptor binding stretch, induced clustering PTPRσ and inactivated it, while CS, which contained much less receptor binding stretch, tended to induce declustering of PTPRσ and activated its catalytic activity. In this study, we showed that Enoxaparin had the same effect as HS. It is previously reported that Chondrotinase ABC and the peptide mimicking PTPRσ wedge domain which are associated with PTPRσ showed promoted functional recovery in vivo, but it has not been clarified whether HS promote functional recovery in vivo. In this study, we showed that Enoxaparin promoted functional recovery after rat model of spinal cord injury.

Figure1