FTY720 Improves Functional Recovery after Spinal Cord Injury via Non-Immunomodulatory Actions

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ABSTRACT INTRODUCTION:
Spinal cord injury (SCI) results in marked poor neuropathology and limited functional recovery despite adequate existing surgical and medical treatments. We have previously shown that the sphingosine 1-phosphate (S1P) concentration in the spinal cord was significantly increased in the location of a contusion injury and brain ischemia (Stem Cells 2007, Stroke 2008). Because S1P receptors are ubiquitously expressed in many organs including the central nervous system (CNS), and S1P produces a variety of responses related to the function of the nervous system, we hypothesize that targeting these receptors may become a candidate therapy for SCI. In this study, we have examined the therapeutic effects of FTY720, an orally available S1P receptor modulator, on a mouse model of SCI and highlight the non-immunomodulatory mechanism by which FTY720 improves secondary injuries after SCI.

METHODS:
Animals, Female C57BL/6N and CB-17 scid/scid mice (9–12 weeks old) were purchased from Japan SLC, Inc. (Shizuoka, Japan) and CLEA Japan, Inc. (Tokyo, Japan), respectively. All animal procedures were approved by the Institutional Animal Care and Concern Committee of Jichi Medical University, and animal care was performed in accordance with the guidelines of the committee.

Mouse model of contusion spinal cord injury. Contusion spinal cord injury (SCI) was induced using an Infinite Horizon Impactor™ (Precision Systems and Instrumentation, Lexington, KY). After anesthesia with isoflurane, the spinal cord segment was exposed by removing the dorsal part of the vertebra, and a contusion injury of the tenth thoracic (T10) spinal cord was induced at a force of 60 kdyn. Postoperative care was performed as previously described (Kimura et al., 2007).

Drug administration. FTY720 (3, 0.3 or 0.03 mg / kg), SEW2871 (an agonist for S1P receptor; 10 mg / kg), or a corresponding control solvent was given orally to mice every 17/Icr scid mice were used for all experiments. FTY720 improved recovery of motor function after SCI in injured spinal cord by FTY720. Recovery was scored by the Basso Mouse Scale (BMS) open-field locomotor rating scale. Recovery of motor function was also quantified by the Rota Rod performance test. Recovery of motor function was also quantified by the Rota Rod performance test. Recovery of motor function was also quantified by the Rota Rod performance test.

Enzyme-linked immunosorbent assay (ELISA), quantitative reverse transcription-polymerase chain reaction (RT-PCR), and test for blood coagulation. Plasma levels of inflammatory cytokines were measured by ELISA. Real time quantitative RT-PCR was performed to measure mRNA expression of inflammatory cytokines in spinal cord. Plasma prothrombin time (PT) and activated partial thromboplastin time (APTT) were also measured.

Infiltration of peripheral blood cells and microglia in spinal cord. The section of spinal cord after SCI was isolated. Total numbers of CD45-positive cells and cells positive for each blood cell lineage marker were determined by flow cytometry.

Vascular permeability. The extravasation of Evans blue fluorescence in spinal cord was quantified by using a microplate spectrophotometer.

Histological analysis. To assess for myelin sparing, staining with eriochrome cyanine was performed, and the area of myelin sparing in the SCI lesion epicenter was quantified. Accumulation of astrocytes was assessed by immunostaining with anti-glia fibrillary acidic protein (GFAP) polyclonal antibody.

RESULTS SECTION:
FTY720 improves functional outcome after SCI in mice. We first examined whether FTY720, an S1P receptor-activating drug, improves locomotor performance after contusion SCI in mice. Administration of FTY720 significantly improved recovery of hindlimb motor function assessed by both BMS score and Rota-rod performance test after SCI, and the effect continued to the end of the analysis. The improvement of motor function was accompanied with histological amelioration as evidenced by changes in the myelin sparing area.

Failure of FTY720 to ameliorate early inflammatory response after SCI. Early inflammation after the SCI is one of a series of important downstream events that includes secondary injuries. Since FTY720 is known to act as an immunosuppressant through its actions on the S1P receptor, we next examined whether FTY720 modulates inflammatory responses after SCI. We confirmed that FTY720 induces lymphopenia, with CD3e-positive T lymphocytes and B220-positive B lymphocytes disappearing from peripheral blood after treatment with FTY720. However, plasma concentration and mRNA expression of inflammatory cytokines in spinal cord after SCI were unaffected by the treatment with FTY720.

FTY720 reduces infiltration of T cells, but not neutrophils and microglia, at the site of SCI. We next assessed infiltration of peripheral blood cells and activation of microglia in spinal cord after SCI. FTY720 suppressed the late infiltration of CD3e-positive T lymphocytes, but not the early accumulation of peripheral neutrophils and microglial activation in injured spinal cord.

Attenuation of vascular permeability and astrocyte accumulation in injured spinal cord by FTY720. FTY720 failed to improve blood coagulation (PT and APTT) and the amount of extravasated blood in contused spinal cord. Conversely, the diapedesis of Evans blue was markedly suppressed with FTY720 at 3 days after SCI. The area of GFAP immunostaining that represents astrocytic reactivity in injured spinal cord was also reduced by treatment with FTY720.

FTY720 promotes functional recovery after SCI even in scid mice. To investigate whether FTY720 improves functional outcome of SCI through immune modulation, we next employed the severe combined immunodeficiency (scid) mice. FTY720 improved recovery of motor function after SCI in scid mice.

SEW2871, an S1P1 receptor agonist, partially mimics the effects of FTY720. We next employed SEW2871, an S1P1 receptor-specific agonist to distinguish whether S1P1 is specifically involved in the actions of FTY720 following SCI. Although administration of SEW2871 ameliorated motor function after SCI, the efficacy of SEW2871 was considerably lower than that of FTY720.

DISCUSSION:
We here demonstrated a therapeutic effect of the S1P receptor agonist, FTY720, in a mouse model of SCI, and examined the mechanism by which FTY720 improves secondary degeneration after SCI. The main biological activity responsible for these actions is believed to be immunological, but our data suggest that non-immunological role(s) of FTY720 are more important in the treatment of SCI. Although we observed a decrease in late T-cell infiltration into the area of injured spinal cord following FTY720 administration, we saw no decrease of any accumulation of neutrophils or microglia, nor changes in the expression of inflammatory cytokines in the earlier phases following SCI. More importantly, FTY720 maintained its efficacy even in scid mice, confirming the importance of immune-independent mechanisms of action of FTY720 in this mouse model of SCI. Among the secondary injury events that occurs after SCI, we observed that FTY720 decreased vascular permeability and astrocyte accumulation in injured spinal cord. In summary, our data suggest that targeting S1P receptors with FTY720 is an attractive therapeutic approach for SCI that prevents vascular permeability and astrogliosis. FTY720 is an oral drug that has shown efficacy in clinical trials for human multiple sclerosis, and was recently approved by the Food and Drug Administration. The safe profile of FTY720 in the treatment of multiple sclerosis encourages us to apply it in a clinical trial for SCI. In addition, strategies targeted at modulating the S1P concentration in injured CNS may lead to new therapeutic approaches towards repairing various CNS disorders.

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