Sphingosine-1-Phosphate (S1P) Receptors Modulate Endogenous Stem Cell Mobilization and Homing for Bone Regeneration

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Introduction: We have previously shown that coating allografts with FTY720, a S1P agonist, increases the rate of critical size defect healing by enhancing homing of host-derived CXCR4+ stem/progenitor cells such as mesenchymal stem cells (MSCs). In this study we show that pharmacological inhibition of S1P3 via VPC01091 significantly increases mobilization of BMSCs into peripheral blood resulting in accelerated bone repair in rat cranial defects. Additionally, MSCs pre-treated with FTY720 exhibit increased migration towards SDF-1, a CXCR4+ ligand and critical component of the bone marrow niche. These findings advocate the significant role of S1P3 in stem cell chemotaxis. We show that treating animals with both FTY720 coated allografts locally, and VPC01091 systematically is beneficial if controlled temporally. We propose that S1P3 receptor antagonists aids in the mobilization of MSCs, while agonists of the same receptor are critical for stem cell recruitment. Thus, suggesting the presence of a push-pull mechanism that is dictated by S1P receptor specific small molecules.

Materials and Methods: Transwell assays were conducted on MSCs treated with FTY720 to assess migration towards SDF-1. 5mm cranial defects were made in 36 nine weeks old Sprague Daley rats, which were divided into 4 groups (n=9). The rats were treated with a systemic dose of 1 mpk VPC01091, FTY720 coated semi-circular allograft, FTY720 coated semi-circular allograft + a systemic dose of 1 mpk VPC01091 or left untreated. VPC01091 was given the day after surgery and 3 weeks post surgery. Hemavet (Drew Scientific) was used to measure the concentrations of blood cells at days 0, week 1 and week 2 (n=6) (data not shown). The amount of bone regeneration was measured bi-weekly with microCT imaging (n=3-9). Flow cytometry was performed according to standard procedures on the tissue harvested from the defect sites at week 3 (n=3), and from peripheral blood at week 6 (n=3). Monoclonal antibodies (Invitrogen, Abcam) for rat CD45, CD11b, CD54, CD11b, CD54, CD90 were used in both cases. Mason’s Trichrome and H&E staining were done for all groups (n=3).

Results:

Fig 1: Micro CT evaluation of bone growth (A), (B); Representative images of control(C) and VPC01091 (D) treated groups.

Treatment with systemic VPC01091 resulted in substantial bi-weekly increase in bone regeneration compared to the empty defect controls (Fig. #1a, 1c, 1d). This group also showed an increase in the % of CD54 and CD90 positive cells (rats MSC markers) in the defect region at week 3 (Fig. #2a) and in the blood at week 6 (Fig #2b). Animals treated with FTY720 allografts showed a temporal response to VPC01091. Initially, they showed lesser bone growth compared to just FTY720 treatment, but the trend reversed after week 4. Hemavet results show an increase in

Fig 2: Flow Cytometry of defect tissue at week 3 (A) and blood at week 6 (B)

Discussion: These results indicate that a systemic treatment with VPC01091 will significantly accelerate bone regeneration in the absence of any local implant. However, the effectiveness of locally released FTY720 to promote healing requires recruitment of BMSCs via S1P3, suggesting that the time of systemic delivery of a S1P3 antagonist is crucial for the body to engage in this push-pull mechanism of endogenous stem cells. This manifests in the fact that the rate of increase in bone volume at later time points is the highest for the group treated with both FTY720 allograft and VPC01091. The presence of an increased number of MSCs both in the blood, and defect region tissue denotes that the cells required for bone healing are being mobilized into the blood, and recruited to the defect site as late as 6 weeks after injury. Thus, this study shows that the rate of bone growth in large defects can be controlled by a combination of S1P receptor specific small molecules in a time dependent manner. The recruitment of CXCR4+ stem/progenitor cells and enhancement of bone defect healing via neovascularization and osseous tissue in-growth can be achieved through selective targeting and activation of S1P receptors.

Significance: We propose the systemic use of a S1P3 receptor antagonist, VPC01091, to mobilize endogenous stem cells in order to increase bone regeneration. Such endogenous stem cell therapy can be used to enhance bone regeneration in instances when there is substantial soft tissue damage and/or exogenous stem cell transplant is not feasible. Endogenous stem cell therapies have been used in various other ailments like cardiovascular infarctions and can prove to be as effective in bone healing.