**Mesenchymal Progenitor Cell Recruitment by Selective Activation of Sphingosine 1-Phosphate Receptors**

Molly R. Tinius,[1] Edward A. Botchwey,[1,2]
[1]University of Virginia, Charlottesville, VA, United States, Department of Biomedical Engineering and [2]Orthopaedic Surgery

**Introduction**

The presence of stem and progenitor cells at the site of an injury is thought to be a critical factor in the capability of a bone fracture to heal and the speed at which it will do so. This work focuses on the recruitment of those cells via the vascular structure, and the biochemical impetus for them to leave the circulation and move into the tissue. Sphingosine 1-phosphate (S1P), a phospholipid which binds to a family of G-protein coupled receptors ($S1P_1$-$S1P_5$), has been shown to participate in the signaling networks which regulate these processes. Through the use of receptor-specific S1P analogs, we can investigate the interactive roles among inflammation, vascularization, and bone remodeling, with the goal of developing regenerative therapies using these drugs.

Platelet-rich plasma (PRP) is an autologous source of platelet-derived growth factors and sphingosine 1-phosphate (S1P) that is obtained by isolating and concentrating platelets from whole blood through the process of centrifugation. Recent studies have shown that PRP's growth factors aid in the regeneration of bone and soft tissue, making it an appealing field of study for tissue engineering. By using S1P receptor-specific agonists and antagonists in combination, and with PRP, it is possible to selectively recruit stromal progenitor cells to an injured area, while reducing the population of inflammatory cells.

**Materials and Methods**

D1 mesenchymal progenitor cells and MC3T3 pre-osteoblast cells were seeded on Corning Transwell membranes (5 μm pore size, 6.5 mm membrane diameter) at a density of $1.52 \times 10^5$ cells per $cm^2$. After a four-hour incubation and adhesion period, the lower well was filled with media containing 10 ng/mL PDGF-BB and 1 μM of S1P or a receptor-selective analog. Cells were allowed to migrate for eighteen hours in an incubator at 37 °C and 5% $CO_2$. Non-migrated cells were removed from the top of the membrane, and the membrane was mounted on a slide using a DAPI-containing mounting medium. The percentage of migrated cells was calculated by sampling approximately 8% of well area, and significance was determined using ANOVA analysis in Minitab 15.

Male C57Bl/6 background mice were anesthetized by ketamine/xylazine and their tibiae were stabilized by insertion of a 27G needle into the intramedullary canal. The needle was clipped flush with the bone and the skin incision closed. Tibial fractures were produced by dropping a weight of 200g from 40cm using a Bonnarens-Einhorn apparatus. S1P receptor-selective drugs were suspended in Matrigel and delivered to the fracture site by a subcutaneous injection. X-rays and tissue sections for histology were obtained at days 3, 7, 14, 21, and 28.

**Results**

S1P is an agonist for all five S1P receptors. Compound 26 (Cpd26) is a selective $S1P_1$ agonist, and leads to a slight increase in cell migration over S1P alone. VPC 01211 is an agonist for $S1P_1$ and antagonist for $S1P_3$, but shows no change as compared with S1P. This may indicate a limited role for $S1P_3$ in mesenchymal cell recruitment.. JTE-013 is an antagonist for $S1P_2$, and in combination with S1P provides the greatest increase in migration.

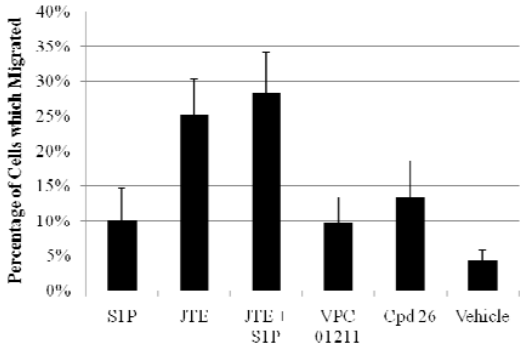


**Fig. 1. S1P receptor agonists and antagonists can be combined to increase progenitor cell migration.** PDGF-BB-induced migration across a membrane was significantly increased ($p < 0.05$) by the addition of an $S1P_2$ antagonist and $S1P_1$ agonists.

These Transwell experiments were repeated using MC3T3 preosteoblast cells and incorporating PRP. Preliminary results indicate a synergistic role for sphingosine 1-phospate-targeted drugs and platelet rich plasma.

At time of submission, control fracture studies are at the day 14 timepoint. These ongoing studies will elucidate the role of S1P receptors fracture healing through loss-of-function studies with knockout animals as well as exploring the potential for S1P receptor-selective drugs for fracture healing therapies.

**Discussion and Conclusions**

Results indicate that S1P receptors 1 and 2 may hold keys to the recruitment of mesenchymal progenitor cells. Specifically, exclusive agonism of $S1P_1$ in combination with antagonism of $S1P_2$ may provide the unique combination necessary to promote tissue healing by increasing movement of these cells out of circulation and into injured tissue. We will investigate these results further with histology of *in vivo* fracture studies to look for the presence of progenitor cells and indications of immune response.

**Acknowledgments**

Support was provided for this research by the National Institute for Dental and Craniofacial Research at the National Institutes of Health, R01DE19935-01.

**Disclosures:** The authors have nothing to disclose.