Enhancement of Bone Regeneration Through Novel VEGF Binding Peptide PR1P

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Purpose: Mechanical stability, revascularization, and osteogenesis are all required for proper bone healing. The optimal stimulation of fracture repairs, particularly in situations of atrophic nonunion, has yet to be discovered. Vascular endothelial growth factor (VEGF) is of particular interest, because of its ability to induce angiogenesis. A newly developed 12-amino acid peptide (PR1P) that binds to VEGF and protects it from proteolytic degradation has shown angiogenic benefits in multiple organs. We hypothesize that PR1P will have enhanced effects during the early stages of fracture healing through upregulation of VEGF and augmented angiogenesis.

Methods: Following IACUC approval, sixteen 9-week-old female mice were subjected to unilateral femoral shaft intramedullary (IM) fixation followed by a fixed traumatic injury to the midshaft. Animals were given 0.1 mL intraperitoneal injections every other day. Treatment groups were saline (control), 100 ug PR1P (A), 5000 ug PR1P (B), or 1000 ug PR1P (C). At 10 days postoperatively, animals were euthanized and tissue harvested for histological staining and qualitative polymerase chain reaction gene expression analysis. One-way analysis of variance or Kruskal-Wallis test followed by post hoc comparisons were performed using GraphPad. Two-tailed values of $P<0.05$ were considered statistically significant.

Results: PR1P binds to VEGF and upregulates VEGFA and its downstream target AKT in fracture healing tissue. Interestingly gene expression for VEGFA and AKT increases with increasing therapeutic dosage, and AKT expression achieves significance at high PR1P dosages compared to control. VEGF receptors 1 and 2 exhibit slightly enhanced expression when compared to controls, especially at higher doses. The bone metabolic indicators SOST ($P>0.05$) and RANKL ($P <0.05$), as well as the RANKL to OPG ratio, demonstrate higher expression with increased dosage, whereas OPG and ALPL remain unaltered.

Conclusion: PR1P stimulation of VEGF has the potential to induce angiogenesis and modulate fracture healing pathways, as revealed by gene expression analysis. PR1P may effectively modulate osteogenic processes when administered at the appropriate dose and time. Increased RANKL levels suggested osteoblastic activity, whereas a skewed RANKL to OPG ratio showed increased osteoclastic activity. Furthermore, RANKL expression during early fracture repair is critical in modulating the immune response. This study provides preliminary insight into potential therapeutic options that can be a part of the bone healing cascade, particularly in nonunion or delayed union cases.

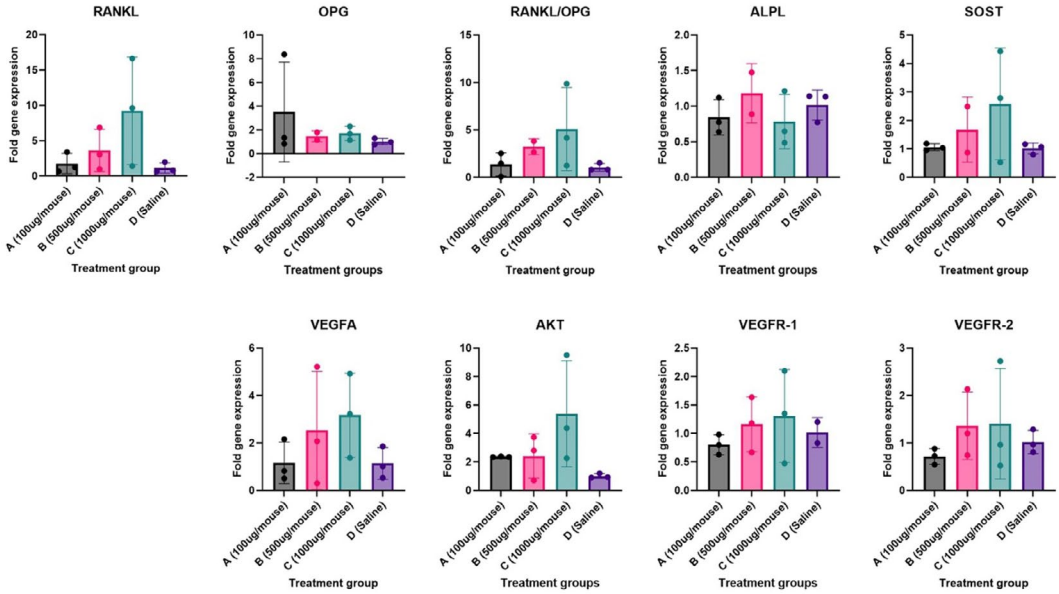

Figure 1A. qPCR data representing gene expression changes compared to Saline control and standardized to housekeeping gene B-actin.
RANKL: Receptor Activator of Nuclear Factor Kappa B Ligand; OPG: Osteoprotegerin; VEGFA: Vascular Endothelial Growth Factor A; AKT: AKT Serine/Threonine Kinase 1; VEGFR-1: Vascular Endothelial Growth Factor Receptor 1; VEGFR-2: Vascular Endothelial Growth Factor Receptor 2; ALPL: Alkaline Phosphatase; SOST: Sclerostin
* P <0.05