Introduction

rhPDGF-BB is a polypeptide growth factor released from platelets at injury sites. It initiates early stage wound healing and is chemoattractive and mitogenic for mesenchymal cells that can differentiate to osteoblasts, chondrocytes, and vascular smooth muscle cells. Moreover, rhPDGF-BB is pro-angiogenic and upregulates VEGF. Consequently, rhPDGF-BB will provide significant therapeutic opportunities in orthopaedic wound healing. Enhanced fracture healing in osteoporotic and geriatric patients is an especially compelling opportunity for rhPDGF-BB, based on its biological properties.

rhPDGF-BB combined with particulate β-tricalcium phosphate has received marketing approval in the United States and Canada for the clinical restoration and regeneration of periodontal tissues, including alveolar bone, periodontal ligament and cementum, and is available commercially under the trade name, GEM 21S®. Another product combining rhPDGF-BB and β-TCP, GEM OS2™, is currently in clinical trials for enhancing the healing of foot and ankle fusions.

Osteoporosis affects over 28 million Americans and leads to decreased bone strength (Harrop et al, 2004). Aging and osteoporosis compromise fracture healing rate and repaired bone quality. The angiogenic, mitogenic and chemoattractive properties of rhPDGF-BB will have a profound and beneficial positive impact on fracture healing in this population. Localizing rhPDGF-BB to the fracture can be accomplished with β-TCP, a commercially available product approved for orthopaedic applications as a bone void filler. Consequently, this study evaluated the use of rhPDGF-BB, delivered within a collagen/β-TCP matrix (Kensey Nash Corporation, Exton, PA) using a well-defined formulation that yields an injectable paste (GEM OS2TM), for enhancing fracture healing in an osteoporotic, geriatric rat model.

Materials and Methods

The study protocol and animal care was approved by the local IACUC and conducted according to AAALAC guidelines at Carnegie Mellon University.

Eighty (80), 6 week old virgin female Sprague Dawley rats were ovariectomized (OVX), placed on a 30% caloric reduced diet for 4 months to ensure osteopenia and then were housed until they were 2 years of age. The rat ovariectomy model used in this study is a well-recognized animal model of compromised wound healing, and approximates the clinical setting of delayed healing in osteoporotic individuals (Walsh et al., 1997). The dosages of rhPDGF-BB tested were 0.3 mg/ml and 1.0 mg/ml in 20 mM sodium acetate buffer, pH 6.0 +/- 0.5. The matrix material (GEM OS2™) consisted of bovine type I collagen and β-TCP with a particle size of 75-300 μm (Kensey Nash Corporation). Immediately prior to surgery, the rhPDGF-BB in sodium acetate buffer or the acetate buffer was mixed aseptically with the matrix material at a ~1:1 liquid to matrix mass ratio to generate a putty-like material. The material was loaded into the barrel of a 1 cc tuberculin syringe and extruded in 18 mm x 2 mm segments that were applied to the fracture site of each tibia.

Rats were assigned to one of four (4) treatment groups: (1) fracture alone (no experimental material), (2) fracture + buffer + collagen/β-TCP, (3) fracture + 0.3 mg/ml rhPDGF-BB + collagen/β-TCP, and (4) fracture + 1.0 mg/ml rhPDGF-BB + collagen/β-TCP. Fractures were transverse, mid-diaphyseal osteotomies prepared surgically using an open procedure in the tibiae. Stabilization was accomplished by placing a 0.7 mm K-wire within the intramedullary canal. A total of 10 animals were included in each treatment group and the time of sacrifice was either 3 or 5 weeks post-surgery.

At 3 and 5 week periods, treated and contralateral untreated tibiae were harvested and the K-wires were carefully removed. Tibiae were selected at random for microCT analysis and fixed in 10% formalin. Following microCT analysis, the tibiae were processed for histology and selected at random for microCT analysis and fixed in 10% formalin. All remaining tibiae were harvested and the K-wires were carefully removed. Tibiae were processed for histology and embedded in methylmethacrylate (MMA). All remaining tibiae (fractured and unfractured) were wrapped in saline-soaked gauze and stored at -20 C to await torsional biomechanics. Destructive torsional testing was performed on a SmartTest testing machine.

Results

Histological results indicated at 5 weeks post-fracture, untreated and buffer-treated control fractures showed minimal bone healing across the fracture with little to no callus formation along the fracture margins. However, for both concentrations of rhPDGF-BB-treated fractures, enhanced bone healing was observed across the fracture site with moderate to marked bridging callus present. No evidence of ectopic bone formation was observed.

MicroCT analysis indicated that β-TCP particles remained around the fracture sites at both 3 and 5 weeks, but there was less material present at 5 weeks compared to 3 weeks. The appearance of the microCT images of the healing fractures treated with PDGF at either dosage suggested that the fracture healing process was normal and confined to the fracture site.

Torsional biomechanical testing was conducted to compare treated fractured tibiae to contralateral untreated tibiae from the same animal. The untreated and the buffer + collagen/β-TCP treated fractures exhibited no significant change in torsional strength between 3 and 5 weeks. In contrast, fractures treated with either concentration of rhPDGF-BB + collagen/β-TCP showed a statistically significant increase in torsional strength at 5 weeks compared to the 3 week time point (p<0.001 after pooling and averaging torsion data from both rhPDGF-BB concentrations). At 3 weeks, all groups demonstrated a significant difference in torsional strength between the fractured and unfractured legs. However, at 5 weeks the rhPDGF-BB-treated fractures were repaired sufficiently such that no significant difference in strength could be discerned between fractured and unfractured legs. Significant differences in strength remained between untreated and buffer treated fractured and unfractured legs at 5 weeks. There were no significant differences in torsional strength between the two rhPDGF-BB dosing regimes used in this study.

Conclusion

The combination of rhPDGF-BB and collagen/β-TCP enhanced fracture repair. A time-dependent healing effect was observed for rhPDGF-BB-treated fractures based on increased torsional bone strength with time. In contrast, untreated and buffer treated fractures did not exhibit a time-dependent increase in bone strength. The lower dose of rhPDGF-BB (0.3 mg/ml) performed as well as the higher dose (1.0 mg/ml) when mixed with the collagen/β-TCP material for enhancing fracture repair. Neither ectopic bone formation nor abnormal bone formation was observed in any of the test groups.

References
