Introduction

PDGF-BB is a basic polypeptide growth factor released from platelets at injury sites . It initiates early stage wound healing and is chemotactic and mitogenic for mesenchymal cells that can differentiate to osteoblasts, chondrocytes, and vascular smooth muscle cells. Moreover, PDGF-BB is pro-angiogenic and upregulates VEGF. Consequently, PDGF will provide compelling therapeutic opportunities in orthopaedic wound healing.

Recombinant human (rh) PDGF-BB combined with particulate β-tricalcium phosphate is FDA approved in the United States and Canada for regeneration of periodontal tissues, including alveolar bone, periodontal ligament, and cementum, and is available commercially under the trade name, GEM 21S®. Furthermore, rhPDGF-BB has been successfully marketed in the treatment of diabetic skin ulcers under the name, Regranex (Johnson and Johnson).

The purpose of this study was to evaluate bone toxicity and surrounding soft tissue responses following multiple injections of rhPDGF-BB near the rat metatarsus and femur. The rhPDGF-BB was administered near the first proximal metaphysis and the femur of rats over 13 days. Radiographic, gross, and histopathological observations were made one day following completion of the injection series and after a period of resolution 6 weeks following the last injection. The outcome revealed neither adverse events in hard and soft tissues nor ectopic calcifications.

Materials and Methods

All experiments were conducted at an AAALAC-approved facility with prior approval of the protocol by the facility’s IACUC review board. A total of 80 rats, including 40 females and 40 males, were divided into 8 groups consisting of 10 animals each (5 M, 5 F). Dose groups received injections of 20 mM sodium acetate buffer, pH 6.0, 10 μg/ml, 30 μg/ml, or 100 μg/ml rhPDGF-BB every other day (ie. day 1, 3, 5, 7, 9, 11, 13) for 13 days. Injections of 0.1 ml were made in the right leg of each animal at the first proximal metaphysis and at the lateral surface of the femur. Body weights were measured and clinical observations were made on a weekly basis. Digital photography of the injection sites were performed on each animal pre-dose and immediately following sacrifice. 4 groups of animals (10 animals per group) were sacrificed one day following the last injection and the remaining 4 groups (10 animals per group) were sacrificed 6 weeks after the final injection. Gross observations of the injection sites were made immediately following sacrifice and digital photographs were taken. The right leg of each animal was dissected during necropsy and fixed in 10% neutral-buffered formalin. The tissues were decalcified, embedded in paraffin, and sectioned to 10 um thickness. Transverse tissue sections were stained with hematoxylin and eosin and Masson’s trichrome stain. Histopathological assessment of the tissues was conducted by microscopic examination of at least 3 tissue sections per injection site from each animal. Tissue reactions in muscle and bone including inflammatory and healing responses, as well as signs of bone resorption, osteolysis, hyperplasia, osteogenesis, fibroplasias and exostosis were graded according to IS010993 and USP guideline on a scale from 0 to 6, with 0 indicating “no reaction” and 6 indicating a “marked reaction”. Bioreactivity ratings in bone and soft tissues were assigned by determining the difference between average scores of test animals in a treatment group and average scores of the negative control group.

Results

Body weights for all animals increased normally during the injection phase of the study (day 1 through 13) except for one female rat (animal #6) which lost ~5% of its mass over two weeks receiving 10 μg/ml doses of rhPDGF-BB. Swelling was observed at the metatarsal injection sites on day 14 in rats receiving rhPDGF-BB (30/30 rats) where none was observed in controls (10/10). Swelling was temporary and the tissues had returned to normal appearance by 8 weeks in all animals. No swelling was observed at the femoral injection sites on day 14. Histology from rats sacrificed at day 14, no soft tissue reaction was evident at either the metatarsus or femur for rats receiving 10 μg/ml or 30 μg/ml rhPDGF-BB. A mild inflammatory reaction at the metatarsus was observed in rats on day 14 which received 100 μg/ml rhPDGF-BB, while no reaction occurred in the soft tissue at the femur. There was no difference in reaction at the bone for doses ≤30 μg/ml rhPDGF-BB at either the metatarsus or the femur. In day 14 animals that had received doses of 100 μg/ml a mild bone reaction was observed consisting of increased osteogenesis and fibroplasias in the outer layers of the cortical bone. No ectopic bone formation was observed in tissue near the femur or metatarsus from any animal. By 8 weeks, all signs of accelerated bone remodeling observed at 2 weeks with the 100 μg/ml rhPDGF-BB dose had resolved and no osteogenic activity was observed.

Discussion

The results of this study revealed that multiple injections of rhPDGF-BB in rats led to a mild acute soft tissue inflammatory response and evidence of bone formation and fibroplasia in the outer layers of the cortical bone at both injection sites. This observation was only in rats receiving the highest dose of rhPDGF-BB at 100 μg/ml. The results were not unexpected given that rhPDGF-BB functions to initiate the wound healing process as a potent chemotactic and mitogenic agent for mesenchymally-derived cell types. After a recovery period of 6 weeks following the final injection of rhPDGF-BB, soft tissues return to normal appearance and the cortical bone appeared normal (Table 1).

Table 1 Summary of Bone Bioreactivity Ratings at the Rat Metatarsus and Femur

<table>
<thead>
<tr>
<th>[PDGF-BB] (μg/ml)</th>
<th>Average Bone Bioreactivity Rating* - Metatarsus</th>
<th>Average Bone Bioreactivity Rating* - Femur</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 weeks</td>
<td>8 weeks</td>
<td>2 weeks</td>
</tr>
<tr>
<td>100</td>
<td>2.9</td>
<td>0.0</td>
</tr>
<tr>
<td>30</td>
<td>1.4</td>
<td>0.0</td>
</tr>
<tr>
<td>10</td>
<td>0.7</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* Average Treated Bone scores – Average Control Bone scores. 0-1.5=No Reaction, 1.5-3.5=Mild Reaction, >3.5-6.0=Moderate Reaction, >6.0=Marked Reaction.

The results of this study at 14 days were consistent data previously reported for rhPDGF-BB (Becaplermin/Regranex) injected repeatedly near the metatarsus and femur of rats (Knight et al, 1998). Moreover, outcome from our study indicated effects of rhPDGF-BB on soft and hard tissues observed at 2 weeks at both sites were completely reversed by 6 weeks.

Conclusion

Multiple doses of rhPDGF-BB for 2 weeks administered near the metatarsus and femur of rats led to an expected and predictable outcome of minimal cortical bone thickening that Remodeled and returned to pre-injection contour by 8 weeks. Further, following a brief period of mild soft tissue inflammation after the 2 weeks of repeated injections, by 8 weeks soft tissue returned to a normal appearance. In addition, no ectopic bone formation was observed. Based on the study outcome reported, PDGF-induced tissue effects are reversible in the absence of further exposure to the growth factor.

References


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● Bone Toxicology Study of Recombinant Human Platelet-Derived Growth Factor-BB (rhPDGF-BB) Injected Locally at the Metatarsus and Femur of Rats

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