rhPDGF-BB Augmentation of New Bone Formation in a Rat Model of Distraction Osteogenesis


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Introduction: Delayed healing is a common complication following distraction osteogenesis. PDGF-BB has an anabolic effect on bone, and previous studies suggest that it may accelerate long bone healing (1,2). This study was performed to evaluate the effect of exogenous rhPDGF-BB, delivered in an injectable collagen paste, on new bone formation during distraction osteogenesis.

Materials and Methods: Following IACUC approval, 83 male Sprague Dawley rats (age 6 mos., wt. 425 ± 35 g) were assigned to one of five treatment groups: buffer (n=16), collagen (n=16), 100 μg/ml rhPDGF-BB (n=16), 300 μg/ml rhPDGF-BB (n=17), and 1,000 μg/ml rhPDGF-BB (n=17). A four-pin monolateral fixator was applied to the right femur of each animal, followed by a periosteal sparing mid-diaphyseal osteotomy. Buprenorphine was administered for pain control and antibiotics were given in the drinking water to reduce the risk of pin track infections. Distraction was initiated on post-operative day 7 and included two 0.17 mm lengthenings per day for 21 days, for a total lengthening of 7 mm (3).

50 μl of the rhPDGF-BB control solutions were injected into the distraction gaps on post-operative days 7, 14, 21 and 28 (5 μg, 15 μg and 50 μg rhPDGF-BB per injection for the 100 μg/ml, 300 μg/ml, and 1,000 μg/ml groups, respectively). The rhPDGF-BB was delivered in a viscous solution of 20mM acetate buffer and soluble bovine collagen (Kensey Nash, Exton, PA). Acetate buffer alone and collagen dissolved in acetate buffer were administered as controls. The progression of healing was followed with biweekly high-resolution plane radiographs (Faxitron, Wheeling, IL). All of the x-rays from a given time point (e.g. day 42) were ranked from least to most healed by two independent reviewers blinded to treatment.

Three animals from each group were euthanized on days 35, 42, 49, 56, and 63. At sacrifice the femurs were removed en bloc and placed in formalin. High-resolution 3-D images (16 μm isometric voxel size) were generated of a 16.5 mm region at the mid-diaphysis via micro-computed tomography (μCT40, Scanco Medical). New bone formation (BV) was calculated from a 6.4 mm (400 slice) segment centered in the distraction gap using the scanner system's built-in image processing software.

After scanning the bones were embedded in paraffin and 6 μm mid-sagittal sections were cut and stained for optical microscopy. The histological slides were graded by two blinded reviewers using a five point grading scale, where 0=no new bone, 1=new bone at cut bone ends, 2=new bone bridging ~1/3 of the distraction gap, 3=new bone bridging ~2/3 of the distraction gap, and 4=new bone bridging entire gap but not united, and 5=clearly united and remodeling.

All data was analyzed using SAS (9.1.3, Cady, NC). General linear modeling (GLM) was used to evaluate the radiographic and histologic data, while mixed linear analysis was used to evaluate the BV data. Intraclass correlation coefficients were calculated on the x-ray ranking and histologic grading data (using the “intraclass” SAS macro by R.M. Hamer, Ph.D., Virginia Commonwealth University), and the BV data was logarithmically transformed to improve normality.

Results: 72 animals survived the study and were available for analysis (n=15 for each rhPDGF-BB treated groups, n=14 and n=11 for the buffer and collagen controls, respectively). Six animals were euthanized after intra-operative surgical complications (i.e. stripping of pin hole or femur fracture), while five of the initial buffer and collagen control animals died from adverse reactions to the analgesic and antibiotics. Consequently, the buprenorphine dose was reduced and the antibiotic was eliminated for the remaining animals.

For the x-ray ranking analysis, the data from the two observers (ICC=0.56) was averaged and the means were analyzed. The mean rank of the 300μg/ml rhPDGF-BB treated animals was higher than that of the buffer and collagen controls on day 28 (p<0.05).

Mixed linear analysis of the BV data revealed statistically significant differences among treatment groups (p<0.0001) and sacrifice time point (p<0.0001), as well as a significant treatment x sacrifice time point interaction (p=0.0395) reflecting the increase in BV with rhPDGF-BB treatment through day 49. (Fig.1) There was no statistically significant difference between the control groups (buffer and buffer + collagen) at any time point, so the data from these groups were combined. New bone formation was lowest in the buffer and collagen controls. BV in the 300 μg/ml animals was higher than the controls on days 42, 49 and 56 (p<0.01 for all), and BV in the 1,000 μg/ml animals was higher than the controls on days 42, and 49 (p<0.002). (Fig.1) There were no significant differences in BV among the five treatment groups on day 63.

Discussion: This study was performed to evaluate the effect of exogenous rhPDGF-BB on bone healing during distraction osteogenesis, and to develop a dose response curve for rhPDGF-BB delivered in an injectable collagen paste. We found a significant increase in mid-consolidation new bone formation with 300 μg/ml rhPDGF-BB treated animals graded consistently higher than all of the other groups, while the 100 μg/ml rhPDGF-BB treated animals graded higher than the buffer and collagen controls, and the 1,000 μg/ml rhPDGF-BB treated animals.

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