Characterization of Growth Factors in two Demineralized Bone Matrix Products

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INTRODUCTION: Demineralized bone matrix based products (DBMs) are commercially available as bone graft extenders for fusion procedures. There are increasing brands of these available every year. Very few of these DBM based products have been evaluated for growth factors and fusion efficacy. Recent studies have shown both intra product variability (due to production lots) and inter product variability (product formulations). Signals from growth factors are the impetus to osteoblast differentiation resulting in de novo bone formation. The most potent of these are the bone morphogenetic proteins BMP-2 and BMP-7. The relationship between posterolateral fusion and BMP-2 assayed from different DBM based product was demonstrated in a previous study of Intergro-DBM (ORS 2007).

Research on the existence of other growth factors PDGF, TGF-β, IGF, VEGF, and FGF present in DBMs are profiled in a recent paper by Wildemann (2006). However, the relationship between the actual growth factor and an outcome of fusion was not evaluated.

The purpose of this study is to assess bone formation as a function of multiple growth factors and their interactions in two DBM based products. Multiple in vitro and in vivo assays were used.

MATERIAL & METHODS: Materials: 10 individual production lots from each of two commercially available DBM based products were used (DBX Putty, Synthes; Allocraft, Stryker). DBM formulations are produced from human donor tissue each lot is a unique donor and product formulations differ in the carriers added.

In vitro methods BMP-2 and BMP-7 concentrations in each of DBM lots were measured using quantitative sandwich enzyme immunoassay (ELISA, Kits from R&D systems Inc, Minneapolis, MN). The same method was employed to assay growth factors PDGF, TGF-β, IGF, VEGF, and FGF. These assays employ the quantitative sandwich enzyme immunoassay. A monoclonal antibody specific for each growth factor was pre-coated onto a microplate. Standards and samples were pipetted into the wells and any growth factor present is bound by the immobilized antibody. After washing away unbound substances, an enzyme-linked monoclonal antibody specific for the correspondence growth factor is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color development is stopped and the intensity of the color is measured.

For in vivo osteoinductive potential: 80 mature athymic nude female rats were used (137-188, avg.170g, Harlan Sprague Dawley, IN). Forty rats for each product tests (n=40 per DBM product). L4-L5 posterolateral intertransverse process fusion was performed with decortication of only the L4 and L5 transverse processes (lamina and facet joints were left intact without decortication). Wounds were well irrigated. Each of 4 rats was implanted with an aliquot (equal to 0.3 cc/side) from one lot (n = 4 rats per each of 10 lots, for a total of 40 rats per product for two different DBM based products). The rats were sacrificed at 8 weeks. High Resolution Radiographs were obtained. The Kappa value was 0.86 indicating excellent agreement between two radiographic coders. Histology was done. Explanted lumbar spines were manually tested for intersegmental motion. Multivariate statistical techniques were used.

RESULTS: In vitro study: Intra product variability values are presented from lowest to highest, For DBX Putty: There were differences in amounts of BMP2 (40.50-96.20 pg/mg), BMP7 (46.55-228.91 pg/mg), TGF-β (4.72-11.12 pg/mg), IGF1 (15.03-32.05 pg/mg), VEGF (0.72-2.15 pg/mg) FGF (0.61-1.70 pg/mg). For DBX Putty, there was no significant differences among the production lots. For Allocraft: BMP2 (36.47-118.56 pg/mg), BMP7 (38.17-184.45 pg/mg), TGF-β (0.61-17.80 pg/mg), IGF (2.45-58.65 pg/mg), VEGF (0.33-1.67 pg/mg) FGF (0.14-1.49 pg/mg).

For DBX Putty, strong positive correlation between IGF and TGF-β (r=0.74, p<0.01), VEGF and TGF-β (r=0.80, p<0.005), VEGF and IGF (r=0.72, p<0.02), and a marginal relationship between IGF and BMP7 (r=0.54, p=0.11).

In vivo: The concentrations of BMP2 and BMP7 were not related to posterolateral fusion in this analysis for either DBM product.

DISCUSSION: There is significant lot-to-lot variability in quantities of BMP2, BMP7, TGF-β, IGF, VEGF, and FGF in DBM based products analyzed. PDGF was undetectable. For both of the products tested, IGF, VEGF, and FGF were highly positively intra correlated, however, they were not related to in vivo fusion. While in one of the products there was a strong relationship between VEGF and BMP7, there was none in the other product tested.

The number of successful fusions as determined by manual testing was low for both of the DBMs tested in this study when compared to the slightly greater number of fusions observed with Intergro (ORS 2007). Manual testing fusion results tended to be low yet radiographic results appeared to identify early fusion, however these data were not used as a proxy for manual testing fusion.

Selection for high levels of appropriate growth factors BMP-2, BMP-7, or other growth factors in DBM based products may optimize DBMs use for spinal fusion. BMP-2 and BMP-7 assays may be used to screen DBM lots for osteoinductive potential prior to clinical use based on a previous study. However, this selection criteria was not validated with use of these other DBM based products. Further study with more DBM based products are necessary to optimize this growth factor selection criteria. A selection for higher quantities, levels, of targeted growth factors could simplify predictability of spinal fusion outcomes and improve the design of DBM based products.

REFERENCES:
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Bae: ORS 2007

Poster No. 610 • 55th Annual Meeting of the Orthopaedic Research Society