WNT3A Achieves Comparable Activation Of Bone Grafts As BMP2, While Avoiding the Side Effects

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Introduction: A growing number of skeletal diseases associated with aging have become a huge biomedical burden to society, and a key-compromising factor in patients’ quality of life [1,2]. Bone grafting is one of the most commonly used approaches to address this problem. However, when autografts are not sufficient to regenerate one’s bones due to aging, developmental signaling proteins such as recombinant BMP2 (rBMP2) can be applied to enhance osteogenic ability. Unfortunately, serious complications have been reported since the approval of this protein’s use in clinics, such as inflammation and ectopic bone formation. We sought potential new molecules to avoid these complications while achieving the therapeutic effect. In this study we compared the efficacy and safety of a potential protein therapeutic, liposomal human Wnt3a (i.e., L-WNT3A), to BMP2 in the same animal model.

Methods: The Stanford Committee on Animal Research approved all procedures. Adult, wild-type, host mice (males, 3-5 months old) were anaesthetized. An incision was made 3mm from the sagittal midline to expose the parietal bone. A circumferential, full thickness defect with a 2mm diameter was created using a Micro Dissecting Trephine; the dura mater was undisturbed. Murine bone marrow was harvested from syngeneic mice, >40 weeks were considered aged. Bone marrow was evenly divided into ~20μL aliquots. Each aliquot was immediately placed into 10μL of Dulbecco’s Modified Eagle’s Medium (DMEM) with 10% Fetal Bovine Serum (FBS) at 37°C for 1h. Aliquots were treated with either L-WNT3A (0.15ng/ul) or rBMP2 (0.15ng/ul and 50ng/ul). Bone marrow aliquots were transplanted to calvarial defects and the skin was closed. After surgery, pain control was guaranteed with scheduled Buprenorphine injections. Mice were sacrificed at multiple time points after surgery; tissue was prepared for analyses using standard procedures. For in vitro analyses, bone marrow cells were isolated from tibia and femur of wild type MICE. Attached and unattached bone marrow were separated with standard culture technics. Mouse MSC were cultured as previously described [3]. Mouse skeletal Stem Cells (SSC) were isolated by FACS sorting of CD105+/CD150+/THY1+/CD45+/kit+ /Sca1+/lin- markers. Human MSC was derived from bone marrow cells that are CD44+/CD73+/CD105+/CD90+/CD4-/CD14-/CD19-/CD34-/CD45-. RNA isolation and qRT-PCR was performed following standard procedures. Statistical results are presented in the form of mean ± standard deviation, with N equal to the number of samples analyzed. Two-tailed Student’s t-test and non-parametric Wilcoxon Test were used to determine significant differences between data sets. Significance was attained at P < 0.05 and all statistical analyses were performed with Graphpad software (Prism).

Results: A much lower dose of L-WNT3A is required to achieve the same osteogenic effect as BMP2. Osteogenic ability of aged bone grafts was enhanced with treatment at a 0.15ng/ul L-WNT3A, gave rise to significantly more bone material compared with PBS treated controls (N=5, figure 1A,B). BMP2 failed to enhance osteogenic ability of aged bone graft at 0.15ng/ul (Figure 1C). At a concentration of 50ng/ul, BMP2 was able to enhance the osteogenic ability of aged bone grafts to the same extent as 0.15ng/ul L-WNT3A (Figure 1D).

We further analyzed the quantity of newly formed bone. In the L-WNT3A treated bone grafts, defects were filled by organized, mature, cortical-like bone tissue in situ. In the BMP2 treated bone graft, we observed an extensive heterotopic ossification that extended superiorly and laterally across the calvaria, in addition to the bone formed in the defect (Figure 1E,F and G). We also observed that the edge of newly formed bone in the BMP2 treated bone graft was immature and rich in acidic polysaccharides, and intertwined with fibrotic tissue (Figure 1H).

We further analyzed the response of bone grafts to L-WNT3A at the cellular level in three dimensions: responding cell type, dose-response and time of pathway activation. In the first dimension, treating different sub populations of cells of bone graft with L-WNT3A indicated only specific stem cell population respond to the treatment by activation of Wnt pathway (Figure 2A). In the second dimension, human mesenchyme stem cell was able to respond robustly to the treatment of L-WNT3A with a very low does, and is directly proportional to the does until reach maximal response. (Figure 2B). In the third dimension, the response of human mesenchymal stem cell to the treatment is first seen in 3 hours, peaks at 12 hours, and then declines over time (Figure 2C).

Discussion: Until 2011, nearly 10% of all bone-grafting procedures used recombinant BMP2 protein (rBMP2), but serious adverse effects are common: meta-analyses demonstrate that rBMP2 causes heterotopic ossification, inflammation, and, in high-dose cases, a significant increase in cancers and yet most or all of these adverse events were not reported in the preclinical models used to test rBMP2 [4,5]. Yet unmet needs in aging associated skeletal diseases and injuries demand effective and safe therapeutics. Here, we present data in a mouse model of an alternative therapeutic with higher efficacy and safety. Autografts from aged mice were treated with L-WNT3A and compared with aged autografts treated rBMP2. A much higher does BMP2 was
required to achieve the same osteogenic effect as L-WNT3A. While the L-WNT3A treated autograft generated new bone in situ in the defect, a considerable percentage of the new osteoid matrix formed was outside the defect site in the BMP2 treated autograft. The new bone formed in BMP2 treated grafts became intertwined with other tissue type such as fibrocartilage. The three dimensional analyses of phymocodynamics shows L-WNT3A specifically targets specific stem cell populations. Pathway activation was quantified and is directly proportional to the amount of ligand added, suggesting that L-WNT3A activates the Wnt pathway in a native ligand-receptor binding manner. Time course response suggests that activation of the Wnt pathway by L-WNT3A is transient and reversible, a potential safety feature.

**Significance:** Until 2011, nearly 10% of all bone-grafting procedures used recombinant BMP2 protein (rBMP2), but serious adverse effects are common: meta-analyses demonstrate that rBMP2 causes heterotopic ossification, inflammation, and, in high-dose cases, a significant increase in cancers and yet most or all of these adverse events were not reported in the preclinical models used to test rBMP2[4,5]. In this study we investigated the efficacy and safety of a potential protein therapeutic, liposomal human Wnt3a (i.e., L-WNT3A) as an alternative to rBMP2.

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**References:**

![Figure 1](image-url)
Pharmacodynamics of L-WNT3A on Bone Graft in three dimensions

Figure 1

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