Selected Scientific Materials

Contents

1. Long-Term Prospective Cohort Study of a Local Osteo-Enhancement Procedure (LOEP) to Treat Proximal Femurs of Post-Menopausal Osteoporotic Women

Authors: James Howe, Bryan Huber, Dominique Favell, Ronald Hill, Mary Bouxsein, Harry Genant

Event:
2017 World Congress on Osteoporosis (WCO)
Location:
Florence, Italy
Date:
March 23 – 26, 2017
Status:
Podium, March 26, 2017
Awards:
Blue Ribbon

Objective: To evaluate the long-term safety and efficacy of a minimally invasive local osteo-enhancement procedure to treat osteoporotic femurs with a proprietary calcium-based implant.

Material and Methods: 12 postmenopausal women (mean age 72, range 56-89) with osteoporosis of the hip (mean T-score -3.1) underwent a minimally invasive LOEP. A proprietary triphasic calcium sulfate/calcium phosphate implant was injected into the left proximal femur; the right served as the control.
Subjects were followed for 5-7 years. Outcomes included medical history review, BMD by DXA, and qualitative assessment by x-ray and CT. Data are reported as mean ± SD. N=12 at 1 and 2 years; N=10 at 5-7 years (2 patients failed to complete radiological evaluations). 6/12 patients received bisphosphonates during some period of the study. Statistical comparisons used paired T-tests. The US Western Institutional Review Board approved the study; all patients provided written informed consent.

Results: Femoral neck BMD in treated hips was significantly greater than contralateral control hips at each time point: 0.88 ± 0.13 vs. 0.52 ± 0.06 g/cm2 ($p < 0.001$) at 1 year; 0.85 ± 0.12 vs. 0.52 ± 0.08 ($p < 0.001$) at 2 years; and 0.83 ± 0.12 vs. 0.53 ± 0.04, ($p < 0.001$) at 5-7 years. Femoral neck BMD in control hips did not change from baseline to 5-7 years ($p=0.30$). X-ray and CT analyses demonstrated the implant material was completely resorbed in all patients by 5-7 years and replaced with bone that integrated with surrounding trabecular and cortical bone. There were no procedure or device related serious adverse events. Six osteoporosis-related fragility fractures were observed: 2 control hips (27 and 44 months), 1 treated hip (40 months), 2 vertebrae (73 months and unknown), and 1 humerus (35 months).

Conclusions: Proximal femurs of osteoporotic patients treated with a local osteo-enhancement procedure (LOEP) and a proprietary triphasic implant demonstrated substantial and sustained BMD increases which corresponded with integrated bone replacing the implant. These results support the safety and efficacy of LOEP to treat osteoporotic proximal femurs, and provide a strong rationale for additional studies of LOEP to enhance femoral strength in patients at high risk for hip fracture

2. Injection of a Triphasic Calcium-Based Implant into Cadaveric Proximal Femurs Provides Immediate Biomechanical Improvement

Authors: Jonathan Shaul, PhD, John Stroncek, PhD, Mary L. Bouxsein, PhD, Dominique Favell, MD, Bryan Huber, MD, James Howe, MD, Ronald Hill, PhD

Event:
DVO 2019, Abstract
DVO Brown

Location: Frankfurt, Germany

Date: March 28th - 30th, 2019

Status: Poster + Podium

Introduction: Current osteoporosis pharmaceutical therapies can reduce hip fractures. However, compliance to treatment is low and therapies require up to 18 months to demonstrate efficacy. Therefore, alternative or complementary approaches to reduce the hip fracture risk are needed. One approach is local enhancement at sites at high risk for fragility fracture such as the proximal femur. The objective of this study was to evaluate the biomechanics of cadaveric femurs following a local-osteo enhancement procedure (LOEP) to place a resorbable triphasic calcium sulfate/calcium phosphate implant material (AGN1) within the proximal femur.

Materials and Methods: 45 pairs of fresh frozen, female, cadaveric femurs were obtained from anatomic gift registries. One femur from each matched pair was injected with AGN1 and the other served as an untreated control. Femoral neck and total hip area bone mineral density (aBMD, g/cm2) and sex-specific T-scores were obtained by DXA. Under fluoroscopy a 5.3 mm lateral entry portal in the femur was created. The proximal femur was manually debrided to loosen fat and marrow elements, which were removed via irrigation and suction. AGN1 was then injected into the proximal femur under low- pressure using a backfilling technique. Distribution of the implant material was assessed using computed tomography (CT). Femurs were mechanically tested in a sideways fall configuration (10° adduction and 15° internal rotation) 24 ± 3 hours following the injection. Failure load, work to failure, and stiffness were calculated from force displacement data. Comparisons were made using a paired t- test, with a p-value less than 0.05 considered significant.

Results: Average donor (N = 45) age was 77.8 ± 8.8 years with an average T-Score of -2.8 ± 1.3. CT imaging showed consistent distribution of AGN1 within the femoral neck and interochanteric region of the proximal femur. For all specimens, femurs injected with AGN1 had a 20.5% higher failure load ($p<0.0001$) compared to the paired controls. Cadaveric femurs were stratified by femoral T-score into normal femurs (N=4), osteopenic (N=16), and osteoporotic (N=45). Osteopenic femurs injected with AGN1 increased failure load 20.6% ($p = 0.00035$). Similarly, work to failure increased 17.4% compared to control femurs ($p=0.047$). Osteoporotic femurs injected with AGN1 had increased failure load 26.0% ($p = 0.00005$), yield load 24.8% ($p = 0.0001$), and work to failure 44.6% ($p=0.0004$) compared to control femurs. Normal femurs treated with AGN1 showed no significant differences in any tested variables. AGN1 treatment did not significantly affect femoral stiffness in any group..

Conclusions: IIn osteopenic and osteoporotic cadaveric femurs, failure load and work-to-failure increased significantly with the injection of AGN1 when tested in a sideways fall scenario. These findings provide evidence that the local treatment of osteoporosis with AGN1 is technically feasible and provides immediate biomechanical improvement. Local osteo- enhancement of the proximal femur with AGN1 is a promising treatment to improve biomechanical properties that warrants further investigation.

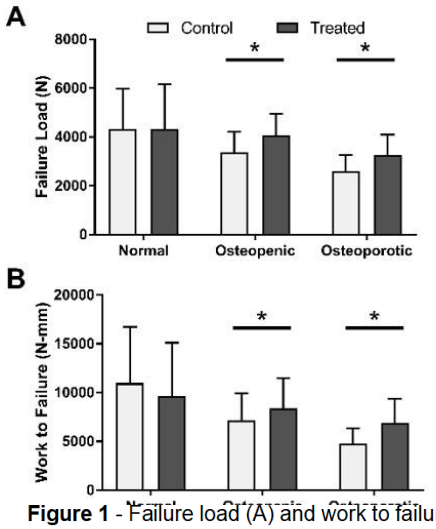

Figure 1 - Failure load (A) and work to failure (B) from all femurs and femurs stratified by T-score. Significant increases ($p<0.05$) were observed in both osteopenic and osteoporotic groups.

3. Triphasic Resorbable Calcium Implant Enhances Bone Formation in Humoral Critical Sized Defects in Canines Independent of Bisphosphonate Treatment

Event:
European Calcified Tissue Society (ECTS) Congress
Location:
Salzburg, Austria
Date:
May 13 – 16, 2017
Status:
Poster

Authors: Ronald Hill, Deborah Hall, Jonathan Shaul, Thomas Turner, Robert Urban
Keywords: Bone, Bisphosphonate, Histomorphometry, Preclinical, Canine

Objective: To evaluate alendronate effects on resorption and biological responses to a proprietary triphasic resorbable calcium sulfate/calcium phosphate implant in critical-sized proximal humeral defects in canines.

Methods: Sixty skeletally mature adult hounds were equally assigned to alendronate (ALN, 0.2mg/kg/day) or vehicle treatment. Unilateral proximal humeral axial defects, 13mm by 50mm, were filled with implant. Intact contralateral humeri served as controls. At 13, 26, and 52 weeks, histology was performed and resorption, bone volume (BV) and microarchitecture were quantified using histomorphometry and μCT. Statistical analysis used Spearman correlations (resorption) and Friedman Tests (BV).

Results: Implant resorption increased over time (r_s=0.524, p≤0.01) with 98.7% resorbed at 52 weeks; the remaining 1.3% was incorporated into trabeculae. Significant neovascularization and macrophage activity were noted during resorption. Defect BV fraction was significantly greater than contralateral controls with ALN at all time points and at 13 and 26 weeks without ALN (Table). In ALN-treated hounds trabecular number and connectivity were significantly higher than controls at all time points and higher than controls at 13 and 26 weeks in hounds not receiving ALN (p≤0.02).

Weeks	13		26		52	
Alendronate	Yes	No	Yes	No	Yes	No
Residual Implant (%)	16.2±18.2	12.2±11.1	4.6±8.8	2.3±1.9	2.7±2.1	1.3±1.5
Treated BV/TV (%)	42.9±7.0*	35.8±12.7*	29.0±16.3*	29.0±11.9*	28.2±7.6*	21.0±9.8
Control BV/TV (%)	14.7±3.9	12.5±1.9	16.1±3.5	15.6±2.0	15.1±2.8	13.4±3.2

* p<0.05 versus control BV/TV

Summary and Conclusions: In critical sized defects in hounds, the calcium sulfate/calcium phosphate implant was resorbed and replaced by newly formed bone with BV fraction and trabecular number and connectivity comparable to or greater than normal control. Alendronate treatment did not influence the response.

4. Injectable Triphasic Biomaterial Resorbs and Increases Bone Formation in Ovariectomized Rat Metaphyseal Defects of the Femur

Authors: Jonathan Shaul, PhD[1], Peggy Lalor, PhD[2], Shane Woods, MS[3], Scott Bruder, MD, PhD[4], Ronald Hill, PhD[1]

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Disclosures: J. Shaul: 3A; AgNovos Healthcare. 4; AgNovos Healthcare. P. Lalor: 5; AgNovos Healthcare. S. Woods: None. S. Bruder: 3A; AgNovos Healthcare. R. Hill: 3A; AgNovos Healthcare. 4; AgNovos Healthcare.

Event:
Orthopedic Research Society (ORS) Annual Meeting
Location:
New Orleans, United States
Date:
March 10-13, 2018
Status:
Podium and poster; chosen as NIRA finalist

INTRODUCTION: Osteoporosis affects 200 million women worldwide and significantly increases hip fracture risk, resulting in a substantial decline in quality of life, an enormous financial burden to the health care system, and a notable increase in mortality rates1-3. In the United States, only 14% of all osteoporotic-related fractures occur in the hip, but this accounts for 72% of all the osteoporotic-related fracture costs2. Bisphosphonates and other systemic therapies have been shown to reduce hip fracture risk. However, significant limitations persist, including a delay in the onset of fracture protection, limited formation of new bone, and low patient compliance4. To overcome these treatment shortcomings, we are proposing a minimally-invasive Local Osteo-Enhancement Procedure (LOEP) involving the injection of a proprietary, triphasic, resorbable, calcium-based implant material (AGN1) to provide immediate mechanical strength and generate new bone to durably strengthen the proximal femur5,6. Previous preclinical studies demonstrated that as AGN1 resorbs it is replaced with new, normal bone in healthy animals7. To evaluate AGN1 in an osteoporotic environment, this study investigated AGN1, its resorption and bone formation in an ovariectomized (OVX) rat model.

METHODS: *Study Design:* Female Sprague Dawley® rats (Charles River Laboratories) were randomly assigned to three groups: Normal Control, OVX Control, or OVX Experimental. OVX was performed at 12 weeks of age, followed by a 12-week bone loss induction period. This time point, 24 weeks of age, was set as "time zero". Retrievals were made at 0, 6, 12 and 18 weeks post-implant. At time zero, 6, and 12 weeks, N = 6 per group; at 18 weeks N = 8 per group. Normal and OVX Control groups without defects were included to evaluate the effect of OVX on bone loss. At time zero, metaphyseal defects, 2.5 mm diameter by 4.0 mm deep, were drilled in the lateral aspect of distal femurs of OVX Experimental animals. Time zero animals received unilateral defects for baseline evaluation. OVX Experimental animals received bilateral defects, one implanted with AGN1, the other an empty control.

Analytical Methods: Radiographs and µCT scans were obtained following removal and fixation of the femur with 10% neutral buffered formalin. µCT resolution was 14.8 µm using a Scanco µCT 100 scanner (Zurich, Switzerland). For each femur, a volume of interest (VOI) was defined to encompass the surgically-created defect. In the groups without defects, a standardized VOI was positioned at a location similar to experimental defects. The percent mineralized volume, mineral density, and trabecular number, thickness, spacing, and connectivity were calculated. Femurs were embedded in methyl methacrylate, and paired thin sagittal sections were cut from the lateral and medial aspects of the defect VOI. Paired sections from 6, 12, and 18-week specimens were stained with H&E and Goldner's Trichrome. Histomorphometric analysis included percent bone and percent residual material. Histopathological analysis included semiquantitative scores for cellular responses. Minitab® 17 (Version 17.3.1) was used for data analysis. Statistical comparisons used ANOVA with Tukey's post-hoc testing with a p-value < 0.05 considered significant.

RESULTS: Bone changes in Control groups (OVX and Normal) were as expected after OVX with 14.9% less bone volume of OVX Controls than Normal Controls at time zero and 27.0% less at 18 weeks. In the OVX Experimental group, AGN1 rapidly resorbed with 2.4 ± 1.9% residual material remaining at 6 weeks and 1.3 ± 1.0 % at 18 weeks. Histology and µCT revealed that residual AGN1 was present as discrete particles on which new trabecular bone had formed. More new trabecular bone filled AGN1 implanted defects compared to empty defects with statistical significance at 6 and 18 weeks (Figure 1). At 18 weeks, trabecular bone architecture in AGN1-filled defects demonstrated increased trabecular number and connectivity, and decreased trabecular spacing compared to empty defects (Table 1). As seen in µCT 3-D reconstructions, in defect VOIs after AGN1 treatment, all cortical wall defects were closed and defects were filled with bridging trabecular bone compared to empty defects, which remained essentially empty with minimal new bone located almost exclusively along the cortical rim (Figure 2). Histopathologic analysis showed no adverse cellular or tissue reactions to AGN1.

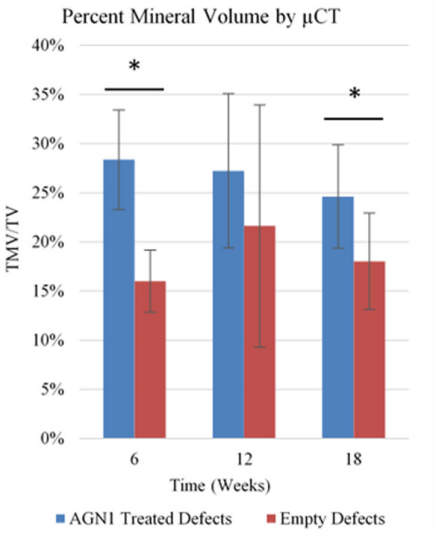

Figure 1 – Percent volume of mineralized tissue within the defects (N=6 for 6 and 12 weeks, N=8 for 18 weeks, * $p < 0.05$).

DISCUSSION: This is the first study that systematically investigated structural and histopathological responses to a triphasic, calcium-based biomaterial in an osteoporosis model. The data demonstrated that with AGN1 treatment of large metaphyseal defects in an osteoporosis model, the cortical wall entry healed completely. As AGN1 resorbed, it was replaced with new trabecular bone displaying a mature architecture. As seen by histology and µCT, residual AGN1 served as a nidus of new bone formation as it was consistently encased in newly formed trabecular bone. This study confirms that AGN1 is resorbed, and then replaced with new bone in a well-established, OVX rat osteoporosis model.

SIGNIFICANCE: For the first time, this study demonstrated that treatment with a unique triphasic calcium-based implant, AGN1, can replace lost bone in an osteoporotic environment, with new bone having a mature trabecular architecture. These findings show that combining AGN1 with a novel minimally-invasive local osteo-enhancement procedure provides a promising new approach to treat bone loss in the proximal femur resulting from osteoporosis.

Table 1 – µCT Quantitative Trabecular VOI Bone Analysis for OVX Experimental Group (* $p < 0.05$).

Time point	Implant	N	Connectivity (per mm³)	Number (per mm)	Thickness (mm)	Spacing (mm)
6 Weeks	Empty	6	24.91 ± 7.91	0.95 ± 0.18	0.16 ± 0.02	1.25 ± 0.27
6 Weeks	AGN1	6	61.37 ± 10.94*	3.14 ± 0.43*	0.13 ± 0.01*	0.32 ± 0.06*
12 Weeks	Empty	6	13.61 ± 9.93	1.31 ± 0.65	0.21 ± 0.01	1.02 ± 0.57
12 Weeks	AGN1	6	25.65 ± 13.03	2.26 ± 0.58*	0.19 ± 0.02*	0.45 ± 0.14*
18 Weeks	Empty	8	9.78 ± 4.57	0.96 ± 0.33	0.21 ± 0.03	1.30 ± 0.44
18 Weeks	AGN1	8	16.88 ± 6.64*	1.85 ± 0.46*	0.21 ± 0.04	0.58 ± 0.27*

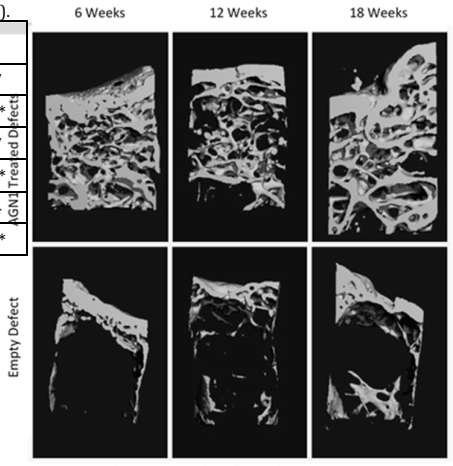

Figure 2 – AGN1 treated defects filled with trabecular bone at all time points (top) compared to empty defects with minimal new trabecular bone (bottom).

REFERENCES: 1. I.O.F. Facts and Statistics. Int. Osteoporosis Foundation (2010). 2. Burge, R. et al. J. Bone Miner. Res. 22, 465–475 (2007). 3. Johnell, O. & Kanis, J. A. Osteoporos. Int. 17, 1726–1733 (2006). 4. Ferrari, S. et al. Arch. Osteoporos., 11, 37 (2016). 5. Shaul, J.L., et al., Trans. ORS, Poster 1342 (2017). 6. Howe, J., et al., Osteoporos Int., 28, S75 (2017). 7. Hill, R.H. et al. Calcif. Tissue Int. 100, S56 (2017).

5. Consistent Implant Resorption and Bone Formation in Three Species With and Without Antiresorptive Therapy Following Treatment with a Triphasic Calcium-based Implant Material

Authors: R. Hill[1], J. Shaul[1], J. Howe[1], D. Burr[2], D. Hall[3], T. Turner[3], R. Urban[3], B. Huber[4], K. Engelke[5], H. Genant[6]

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Event:
WCO 2019,
Abstract
Alendronate_1335

Location: Paris, France

Date: April, 4th - 7th 2019

Status: Poster

Objective: The study compared implant resorption and bone formation in 3 species with and without antiresorptive therapy following triphasic calcium-based implant material (AGN1) implantation.

Materials & Methods: Three species were studied, each with subgroups receiving either antiresorptive treatment or none: drill hole defects in OVX rat proximal femurs (N=6/group) and dog humeri (N=10/ group), and proximal femurs in 12 osteoporotic women. Patients had one proximal femur injected with AGN1 as part of a local osteo-enhancement procedure (LOEP); the other was an untreated control. Half were prescribed antiresorptives. Analyses included µCT/histopathology (rat/dog), biomechanics (dog) and DXA/CT (patients). Follow-up timepoints for rats, dogs, and patients were 18, 26, and 25 weeks.

Results: More than 95% of AGN1 was resorbed by 26 weeks in all species; resorption was not significantly affected by antiresorptive treatment. AGN1 was replaced by normal bone in rats as assessed by histology/µCT and in dogs by histology/µCT/biomechanical testing. In patients, AGN1 increased bone in the proximal femur as assessed by CT and femoral neck aBMD (0.917±0.140 vs control 0.530±0.045 g/ cm2, p<0.001). In rats, alendronate (15µg/kg 2x wk) increased percent bone (26.4±14.0% vs 13.4±6.0%, p=0.012). In dogs, alendronate (0.2mg/kg/day) had minimal impact on percent bone (22.0±5.2% vs 19.0
±3.4%, p=0.147). In patients, antiresorptives did not significantly impact aBMD (0.938±0.152 vs 0.897
±0.139 g/cm2, p=0.636).

Conclusions: The study showed antiresorptives had no significant effect on AGN1 resorption across species. Normal bone formed in rat and dog implanted defects regardless of antiresorptive treatment. The good bone quality in the preclinical models and similar patient results suggest that new patient bone would also be healthy and metabolically active. These results demonstrate that the effectiveness of AGN1 LOEP as a treatment for local osteoporotic bone loss is not compromised by antiresorptive treatment.

We hope you enjoyed this selection of AgNovos' scientific material. For more information about the research supporting OSSURE LOEP, please contact medicalaffairs@agnovos.com