

Enhancing peri-implant bone formation using a combination of synthetic hydroxyapatite and systemic bisphosphonates: A translational study from experimental animal studies to humans

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INTRODUCTION: Securing fracture fixation hardware using screws and pins in osteoporotic bone can be challenging. We previously demonstrated that an injectable ceramic biomaterial comprising of calcium sulphate (CaS) and hydroxyapatite (HA) applied at the interface of a metallic screw and osteoporotic bone could; 1) enhance the immediate mechanical anchorage of the screw and 2) the biomaterial could be biologically activated by systemic administration of a bisphosphonate, zoledronic acid (ZA)¹ leading to increased peri-implant bone formation in preclinical models. Two important questions to explore are 1) the timing of ZA administration and its role on peri-implant bone formation and 2) whether the concept can be translated into a clinical setting.

METHODS: *Pre-clinical study 1:* The first study was performed in 25 male Sprague-Dawley rats. 20 rats received a CaS/HA biomaterial in a burr hole (diameter 1.8 mm) in the proximal tibia in which a stainless-steel screw (diameter 2.3 mm, height 8 mm) was inserted. The remaining 5 animals did not receive the CaS/HA material. The first batch of 20 animals were divided in four groups of five rats. 1) Injection with fluorescent ZA (FZA, labelled with Alexa Fluor 647) on day 1, 2) Injection with FZA on day 3, 3) Injection with FZA on day 7, and 4) Injection with FZA on day 14. The remaining 5 rats, which did not receive the CaS/HA material were also injected with FZA on day 14. FZA was administered as a single dose sub-cutaneously (0.1 mg/kg). 24 h after the FZA injection, the animals were sacrificed and their tibiae scanned in an In-Vivo Imaging System (IVIS Spectrum, Perkin Elmer, USA). The fluorescence signal in the burr hole region and in the anatomically equivalent region on the contralateral tibia was quantified. *Pre-clinical study 2:* In the first pre-clinical study, we could not elucidate whether timing of ZA administration affects the consequent peri-implant bone formation at a later time point since the animals were sacrificed 24 h after ZA administration. To achieve this study goal, we therefore repeated the implant integration model using male Sprague-Dawley rats (average weight= 429±50 g). A total of 35 rats were divided into five experimental groups (n=7/group) as follows; G1) CaS/HA, G2) CaS/HA+ Systemic ZA on day 1, G3) CaS/HA+ Systemic ZA on day 3, G4) CaS/HA+ Systemic ZA on day 7 and G5) CaS/HA+ Systemic ZA on day 14. In groups G2-G5, all animals received one sub-cutaneous injection of ZA (0.1 mg/kg). A control group without CaS/HA material around the screw was also used with 4 rats. The control group animals received ZA (0.1 mg/kg) 14-days post implantation, based on earlier reports². All animals were sacrificed 6-weeks post-surgery. Tibiae implanted with the metal screw were harvested and subjected to micro-CT imaging to quantify peri-implant bone formation. *Clinical study:* A total of ten patients were included in this pilot study and were part of a larger randomized study. All patients were osteoporotic and had a low energy trauma causing a trochanteric fracture. All patients were treated with a dynamic hip screw (DHS); 1) DHS+Systemic ZA at 1-2 weeks post-surgery (N=5) and 2) DHS+CaS/HA+Systemic ZA 1-2 weeks post-surgery (N=5). 2.5 mL of CaS/HA was applied at the tip of the DHS using a special technique we described in an earlier study¹ before placing the DHS at its final position. At 6-months post-surgery, DXA based BMD in a defined ROI was used as a primary outcome variable. CaS/HA material was purchased from Bonesupport AB, Sweden. The animal experiments were approved by the Swedish Board of Agriculture (15288/2019). The clinical study was approved by the Institute Review Board of the Kaunas University Hospital, Lithuania (P1 BE-2-76/2019).

RESULTS: *Pre-clinical study 1:* Compared with the control group (i.e. no CaS/HA but systemic ZA at 2-weeks), the uptake of FZA was significantly higher in all CaS/HA groups irrespective of the time of ZA administration, elucidating the importance of peri-implant HA presence. When comparing the CaS/HA groups, the uptake of FZA was highest in the day 7 group, which was significantly higher than day 1 or day 14 groups. *Pre-clinical study 2:* Compared with the control group (i.e. only CaS/HA, no ZA), the peri-implant bone volume (BV) was significantly higher in the day 7 and day 14 groups. In agreement with the pre-clinical study 1, BV was highest in the day 7 group, when compared with day 1 or day 3 groups. *Clinical study:* At the 6-month DXA follow-up, a 12% increase in the BMD could be observed in ROI R1 in the treatment group in comparison with the control group (i.e. only systemic ZA). In ROI R2 and R3, 15% increase in the BMD was observed when compared to the control group. An overall BMD increase (R1+R2+R3) of 14% could be observed in patients treated with the proposed method when compared to standard of care.

DISCUSSION: Failure of internal fixation of fractures in osteoporotic bone typically occurs through breakage of the cancellous bone that surrounds the screw head, and augmentation of the bone at the interface could be considered in patients with trochanteric fractures. The rate of failure that requires revision surgery in trochanteric fractures is about 3-7 %, depending on the fracture type, and the one year mortality in reoperations is as high as 40%. In this study, we used two commercially approved products to improve the implant anchorage mechanically, followed by pharmaceutically modulating the biomaterial, which led to an increased biological fixation. This is the first study, to our knowledge that emphasizes the optimal timing of ZA administration and the uptake in a synthetic ceramic material. By injecting ZA during the optimal time window of 1-2 weeks, a positive effect on peri-implant BMD has also been seen in our limited patient series. In order to further verify the clinical efficacy of the developed approach, a larger randomized controlled trial is necessary before it can become regular clinical practice.

CLINICAL RELEVANCE: By enhancing the mechanical and biological anchorage of metallic hardware to osteoporotic bone, the rate of revision surgery could potentially be reduced, which could aid in reducing the consequent morbidity and mortality associated with hip fractures.

REFERENCES: 1. Raina et al., Frontiers in Bioeng and Biotech, 2022. 2. Amanat et al., J Bone Miner Res, 2007.

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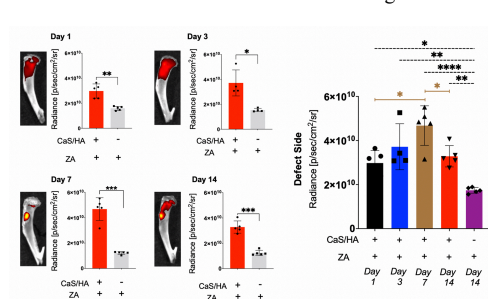


Figure 1: Results from the pre-clinical study 1 showing the uptake of FZA in a pre-implanted CaS/HA material as a function of injection time.

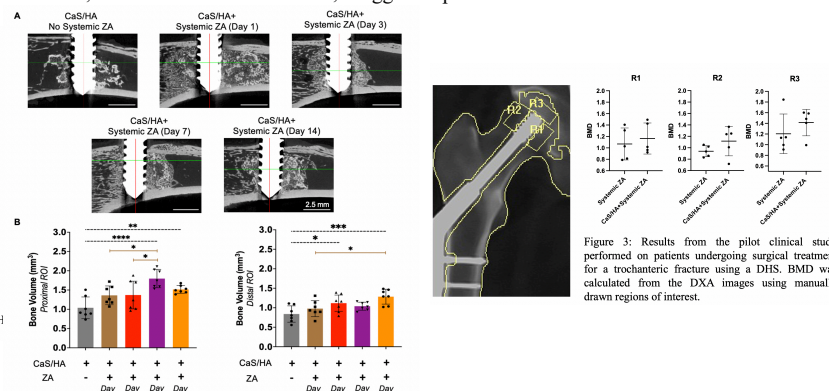


Figure 2: Results from the pre-clinical study 2 showing the relationship between ZA administration time and the eventual effect on peri-implant bone formation.

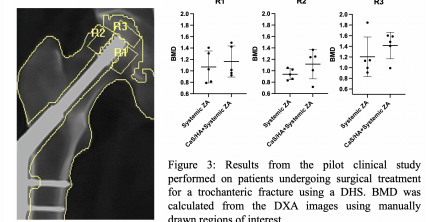


Figure 3: Results from the pilot clinical study performed on patients undergoing surgical treatment for a trochanteric fracture using a DHS. BMD was calculated from the DXA images using manually drawn regions of interest.