Combining systemic and local osteoporosis treatments: a longitudinal in vivo microCT study in ovariectomized rats

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Disclosures: V.A. Stadelmann: 3B; paid consultant Flowbone. E. Gerossier: None. U. Kettenberger: 3A; Flowbone. 4; Flowbone. D. Pioletti: 4; Flowbone.

INTRODUCTION: The management of osteoporotic patients diagnosed with imminent risk of fracture poses a significant challenge as anti-osteoporotic drugs have a slow onset of action and show limited effectiveness in regions like the proximal femur. To overcome these limitations, a possible strategy for fracture prevention involves combining systemic anti-osteoporotic therapy with local bone augmentation. In this study, a novel, highly injectable, hydrogel-based biomaterial for local bone strengthening was used together with systemic anti-osteoporosis treatments and a local bisphosphonate to asses combined effects on bone density in-vivo in a rat model of osteoporosis.

METHODS: This study was approved by the local ethical committee. 36 ovariectomized (OVX) Wistar rats were randomly assigned to the vehicle (VEH), alendronate (ALN) or parathyroid hormone (PTH) systemic treatment group. Systemic treatments started immediately thereafter until the end of the study: VEH received 1ml/kg NaCl 0.9% 2×/week, ALN was administered 2×/week, 20 µg/kg body weight, PTH (rhPTH 1-34) 3×/week, 5.6 µg/kg. One week later, the 72 tibias were block-randomized into 3 local treatments sub-groups (NaCl, biomaterial, biomaterial-ZOL), ensuring that no animal received the same treatment in both legs. The biomaterial (flowbone, Switzerland) or NaCl was injected bilaterally under general anesthesia. For this purpose, a 0.3mm unicortical hole was created in the medio-proximal aspect of the tibia, 2 mm distal of the growth plate, and 10µl of the test material (provided read-for-use by the manufacturer) were injected into the trabecular bone with 0.3mL insulin syringes. MicroCT scans of the proximal tibiae were acquired at baseline (week -1), immediately post-injection (week 0) and at 2, 4, 6 and 8 weeks thereafter with a preclinical in vivo scanner (VivaCT80, SCANCO Medical) under general anaesthesia.
The injection volume of interest (VOI) was identified in post-op scans by segmenting the contrast-agent containing biomaterial from its surrounding, based on its high contrast. The contrast agent was washed out shortly after injection, hence not influencing the quantification of mineralization and bone growth in subsequent microCT scans. For the tibias injected with NaCl, which is not discernable from tissues, generic injection VOIs were automatically generated, encompassing half of the trabecular bone region around the injection point, totaling a volume equivalent to the injected biomaterial. The follow-up scans were registered to the post-operative scans, and the injection VOIs propagated onto all subsequent time points. The trabecular bone within and surrounding the injection VOIs was analyzed separately. Image processing was performed using EasyIPL (easyipl.com), a high-level library of macros using the scanner software (SCANCO Image Processing Language IPL and OpenVMS Digital Command Language). After euthanasia, bone samples were harvested and embedded in PMMA for histological analysis.

RESULTS SECTION: As expected, a significant bone loss was observed in control tibia where VEH was used as systemic and local treatment. The systemic treatment with ALN was found to completely prevent bone loss, while PTH only slowed it down. In VEH animals, the local treatment with biomaterial injections increased bone mass by 110%, but the effect was transient and bone mass returned to baseline values by week 8. In ALN treated animals, local biomaterial increased bone density within the injection VOI by 260% at week 4, in BMD and BV/TV, but the newly formed bone resorbed continuously after the peak, at a rate of 6% / week. Tibias injected with ZOL-loaded biomaterial and treated with ALN systemic showed a gain in bone by 320% at week 4, followed by a slower decay of 3% / week (Fig. 1). Bone outside the injection VOI was not influenced by the injected material.
When combined with systemic PTH, a combined effect was less clear. Additional investigations will be necessary to confirm a combined effect between systemic PTH and local ZOL integrated into the biomaterial. Our data also shows that at baseline BV/TV of the injection VOI was lower than in the surrounding bone, confirming that the injected biomaterial penetrates into regions of reduced density where resistance is lower. Histological results from week 8 revealed a dense network of newly formed bone with integrated mineral remnants of the injected material. The structural features of the mineralized tissue appearing on the microCT sections suggest that both biomineralization of the injectable biomaterial and new bone formation, occurred simultaneously.

DISCUSSION: This in vivo rat study showed that a local treatment with the novel, injectable biomaterial with and without ZOL supplementation increased bone mass within the injected VOI. When combined with systemic antiresorptive treatments, the bone gain was made more sustainable. The marked difference between the ALN and PTH systemic treatments was unexpected and may be attributed to the chosen drug doses or the specific animal model used for this study. The longitudinal preclinical model used for this study has been shown to be well suited for the monitoring of region-specific bone changes. No effect on the bone outside the injected zone was observed with ZOL-loaded biomaterial, suggesting that ZOL remains highly localized and is not released into the surrounding bone at the low dose used in this study. One limitation of the model is that as the animals grow, the cortical injection hole migrates away from the growth plate towards the diaphysis artificially increasing the apparent bone loss.

SIGNIFICANCE/CLINICAL RELEVANCE: This study suggests that an injectable biomaterial capable of promoting new bone formation could be a valuable addition to established anti-osteoporotic drugs and can be used in combination with these drugs. It has the potential to increase bone density rapidly and significantly in regions identified as at high risk of fracture.

Fig 1. (Left) Series of registered uCT sections at the injection points* for the groups: OVX, systemic ALN, injectable biomaterial (IB), combination of systemic ALN + IB-ZOL. Injection VOIs are shown orange (without IB, generated empirically) or green (with IB, based on segmentation). (Right) Time series of BV/TV for the same groups. Shaded areas represent the 95% confidence intervals (CI) of the data fitted with general additive models.