A Single Local Dose Of Erythropoietin Augments Calvarial Bone Healing In 18 Pigs
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Disclosures:

Introduction: Erythropoietin (EPO) has been demonstrated to exert an osteogenic action in cell studies and small-animal models [1-3]. So far, the applied supra-physiological doses of EPO and the fact that no data from large-animal models exist, have stalled the clinical progress. The determination of a clinically safe dose that still enhances bone formation is therefore a necessity for clinical translation. Our hypothesis was that a single local dose of EPO augments bony ingrowth in a porcine calvarial defect model. Furthermore, we hypothesized that the combined dose of 2700 IU EPO per animal does not have a systemic effect assessed by means of hematological quantities.

Methods:
Female Danish Landrace pigs (n = 18, 193 ±11 days, 146 ±16 kg) were used in this paired, block-randomized study to compare six groups in a cranial bone defect model. The within-subject design eliminated the variability caused by biological variation among animals. A sample size calculation determined the number of animals (n = 18). The six treatment groups consisted of autograft ± EPO, collagen scaffold ± EPO, and polycaprolactone (PCL) scaffold ± EPO (Figure 1).

In each pig, six cranial defects (Ø 10 mm, depth 10 mm) were drilled into the skull. The periosteum was stripped. Subsequently, the six 0.785 cm³ defects were filled either with cranial autograft (0.701 ±0.005 g), a porous fusion-deposition modeled PCL scaffold, or a commercially available collagen carrier (Sangustop®, Aesculap AG, Tuttlingen, Germany) in conjunction either with 900 IU EPO (epoetin beta, NeoRecormon®, Roche, Welwyn Garden City, UK) or an equal volume of saline. The saline collagen group resembled the negative control group and assessed the intrinsic healing capacity. After an observation time of 5 weeks the primary outcome evaluation, bone volume fraction within the defect (BV/TV), was assessed with high-resolution quantitative CT (qCT, voxel size = 82 µm³). The images were analyzed with the ImageJ-plugin BoneJ [4]. Secondary outcome evaluations were histomorphometric analysis of the BV/TV, fibrous tissue, blood vessels, scaffold and artifacts. Two blinded observers applied stereological principles and point counting technique. The intra- and inter-observer variation was determined and expressed as coefficient of variance (CV%). In order to record a potential systemic effect of EPO, hematological quantities at baseline, 1, 2, and 5 weeks were determined.
All experiments were conducted at least twice with six technical replicates. Normally distributed data are given as mean ±
standard deviation and were analyzed with one-way ANOVA and Fisher’s post hoc testing against the positive control, while skewed data are reported as median (interquartile range) and were analyzed with Friedman repeated measures analysis of variance. Statistical significance is reported when p ≤ 0.05. The experiments were approved by The National Authority, no. 2012-15-2934-00362.

**Results:**
One animal was killed five days post-operatively due to reasons unrelated to the cranial surgery. The remaining 17 animals (157 ± 14 kg) corresponding to 102 bone defects and 68 blood samples were available for analysis. The median BV/TV ratio was 1.06 (1.01-1.10) in the EPO group relative to the control collagen group (p = 0.001). In agreement with this observation, histomorphometric evaluation documented trends towards increased bone healing, improved vascularization and reduced fibrous tissue formation in EPO-treated compared with saline-treated defects (p > 0.05, Figure 3). The inter-observer CV% was 5% and the intra-observer CV% was 2% regarding BV/TV.

The excellent healing capability of autograft with or without EPO was exhibited, and the BV/TV did not statistically significantly differ from the adjacent calvarial bone, which defined a reference (Figure 2, ref.). In contrast, the bony ingrowth into the PCL scaffold was sparse both with and without EPO. In fact, the saline-loaded collagen (negative control) performed better than the PCL scaffold groups (Figure 2).

The systemic effect of EPO was negligible, if present at all. In brief, taking multiple comparisons into account, there were no statistically significant differences compared with baseline. However, a minor tendency towards increased HGB was observed. The largest observed HGB difference was 0.68 ±1.10 mmol/L, which was seen when week 5 was compared with baseline (p = 0.022). Importantly, no adverse events were observed.
Discussion: To the best of the authors’ knowledge this is the first study to investigate the osteogenic potency of EPO in a large-animal study. A single topical dose of EPO augmented bony ingrowth (BV/TV) when applied on a collagen carrier. The median effect size of 6% (1-10%) was slightly smaller than the observed effect after higher, continuous dosing in small-animal models (1, 2). However, the applied dose was considered clinically safe because we did neither observe a systemic effect nor adverse events. In previous small-animal studies, an increased neovascularization was reported and suggested to be partly responsible for the improved bone healing (1,2). In contrast to these reports, no statistically significant difference in vascularization was observed in the present study. Neovascularization is therefore unlikely to cause the enhanced calvarial bone healing after EPO-treatment.

Given the moderate effect size, further large-animal studies that aim to optimize the effect size without compromising the clinical applicability of the treatment regime are required before clinical trials should be commenced. Moreover, the ability of EPO to increase bone healing in more clinically relevant settings, for instance a fracture model of the long bones or a spinal fusion model, remains to be tested in a large-animal model.

Significance: This is the first large-animal study to investigate the osteogenic efficacy of EPO. A clinically safe dose of EPO increased the median bone volume fraction by 6% (1-10%). This dose could potentially be tested in clinical trials, for instance in fracture patients prone to impaired bone healing is common and a high risk for atrophic non-unions. However, given the moderate effect size in the present study further dose optimization in large-animals may be needed.

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References:

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