Non-viral delivery of BMP-2 plasmids with Copolymer-Protected-Gene-Vector (COPROG) incorporated in a Poly(D,L-lactide) coating to promote fracture healing

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Introduction: Envisioned as a means of correcting genetic defects, gene therapy has been used for the delivery of growth factors or cytokines to sites of tissue regeneration. The strategy in this case is to accelerate or induce a natural biological process by expressing a molecule that is normally involved in the regenerative response for the tissue of interest. In previous studies the stimulation of bone healing using different recombinant growth factors locally applied from a biodegradable Poly(D,L-lactide) (PDLLA) implant-coating was investigated and a positive effect on fracture healing was shown in vivo (Schmidmaier et al., Bone 2001).

The in vitro transfection of osteoblasts with Bone Morphogenetic Proteine-2 (BMP-2) plasmid delivered by a newly developed, non-viral Copolymer Protected Gene Vector (COPROG) incorporated in a PDLLA coating for orthopedic implants has already been demonstrated (Schwabe et al., Trans Orthop Res Soc 2005).

Objective of this study was to investigate the effect and safety of the local application of the above mentioned BMP-2 plasmid-vector formulation in a rat fracture model.

Materials and Methods: Gene vector: Copolymer Protected Gene Vectors (COPROGs) were synthesized as previously described (Finsinger et al., Gene Ther 2000).

p55pCMV-IVS-Luc+ as reporter gene.

Operation: A standardized closed fracture of the right tibia of five months old Sprague Dawley rats (n=20/group I-III, n=25/group IV) was performed with a fracture device (Schmidmaier et al., EJT 2004). The fractures were intramedullary stabilized using uncoated versus coated titanium K-wires. The animals were sacrificed at 28 and 42 days in groups I-III, respectively at 2,4,7,28,42 days for the luciferase Group (IV).

Following groups were investigated:
I. Control group
II. PDLLA + COPROGs
III. PDLLA + COPROGs + BMP-2 Plasmid (40 μg)
IV. PDLLA + COPROGs + LucPlasmid (40 μg)

Radiology: X-ray examinations (p.a. and lat.) were performed throughout the whole experimental period.

Biomechanical Testing: After sacrifice both tibiae were dissected for biomechanical torsional testing using the Zwick 1455 material testing machine (Ulm, Germany).

Histomorphometry: 5 μm longitudinal sections were cut and stained with Safranin O/Lightgreen and von Kossa. Histological parameters of the fracture callus were measured using the Zeiss KS 400 image analysis system.

PCR: After sacrifice of the animals in group IV (n=5/timepoint) rt-PCR analysis was performed to detect luciferase expression in the bone to prove local transfection. To measure possible systemic expression, different organs (brain, lung, liver, spleen, kidney, ovaries, muscle) of each animal were tested for luciferase expression.

Results: The radiological examination demonstrated expanded callus morphology in the BMP-2 plasmid treated group compared to the control groups at day 42 after surgery.

While the local application of BMP-2 plasmid showed no significant differences between the groups in the biomechanical testing after 28 days, a significantly (p<0.05 ANOVA, Bonferroni) higher maximum load could be detected after 42 days compared to the control group.

The histomorphometric analysis revealed a significantly (p<0.05 ANOVA, Bonferroni) smaller Periosteal Callus Density in the BMP-2 group compared to the control group at day 28. This difference was not seen at day 42. No difference was found at both time points for the other parameters including Tibial diameter, Mineralized Cortex Area, Periosteal Callus Area, Periosteal Cartilage Area Histomorphometric Analysis

Discussion: Generally, viral carriers show a better efficiency but they bear the risk of immunological problems and possible long term complications. Non-viral vectors are advantageous in these terms and they may be a safer alternative to viral vector formulations.

Now, for the first time, a non-viral BMP-2 plasmid application from COPROGs coated implants showed an effect on fracture healing in vivo. The implant serves as fracture stabilisation device on the one hand and on the other hand as a plasmid delivery system. The PCR analysis showed a luciferase expression in all bone specimens from day 2 until day 42. Furthermore the transfection may be considered safe since it occurs only in the tissue of interest. No luciferase RNA was detected in any other organ at any time point, which excludes a systemic transfection.

The biomechanical results showed a significant higher maximum load compared to the control group at day 42, but then the histomorphometric results could not support these findings. At day 28 the Periosteal Callus Density was even significantly lower in the BMP-2 group compared to the control group. This was not found at day 42. This humble effect may be due to the low concentration of the plasmid (40 μg). It is known that transfection efficiency is generally lower for non-viral vectors compared to viral vector systems. So far the COPROGs exactly underline this issue, they are safe but in terms of efficiency they lack behind. But since this is the first in vivo study using the COPROG vector, there is an ample scope to improve the efficacy by charging the COPROGs with a higher plasmid concentration.

* The rt-PCR Analysis was positive for the bone. Luciferase was detected at RNA level in all bone specimens at all time points. On the other hand no luciferase expression could be detected in any other organ at any time point.

** The histomorphometric analysis revealed a significantly (p<0.05 ANOVA, Bonferroni) smaller Periosteal Callus Density in the BMP-2 group compared to the control group at the same time point.

p<0.05 (ANOVA, Bonferroni) significant difference to Control group at the same time point.