

The influence of TGF- β 3 on bone regeneration in an ovine tibia defect - qualitative and quantitative histology -

S.Terhorst¹, U.Schlegel¹, T.Arvinde², M.Glatt², O.Maissen¹, BA.Rahn¹

¹ AO Research Institute, Davos, Switzerland. ² Novartis Pharma, Basel, Switzerland.

INTRODUCTION: The transplantation of autologous bone is often required for the treatment of large segmental bone defects. It can cause donor site morbidity and is of limited availability [1]. A possible approach is to design an artificial bone substitute, which ideally should stimulate cell differentiation, guide bone formation and provide cells which produce bone matrix [2]. The goal of this study was to investigate the osteoinductive potential of the Transforming Growth Factor- β 3 (TGF- β 3), which is known to regulate functions associated with bone formation [3].

METHODS: Cortical segmental defects of 18 mm length were created in ovine tibiae and treated with: (i): autologous cancellous bone, taken from the iliac crest, n=4; (ii): Poly (L/DL – Lactide) 80/20% sponges (PLA) [4] loaded with recombinant human TGF- β 3 in a concentration of 50 μ g/cm³, n=4; (iii): PLA only, n=4; and (iv): 2 defects were left empty. For stabilization of the defects with full load bearing external fixations were used. After 4 and 6 weeks calcein green and after 8 and 10 weeks xylenol orange were administered for *in vivo* labeling of bone formation. After 12 weeks undecalcified specimens were embedded in methyl methacrylate. 100 μ m thick sections were examined with contact radiography and fluorescence microscopy to measure the activity and amount of mineralization with Zeiss KS 400.3 and image pro plus 3.0. In the operator based qualitative evaluation of Giemsa/Eosin stained sections with bright field light microscopy points of interest were specified on samples, representing bone regeneration, bony bridging, vessel formation, soft tissue in-growth and foreign body reactions. For statistical analyses the Kruskal Wallis test and the Wilcoxon rank sum test were used ($p \leq 0.5$).

RESULTS: Contact radiographs showed the most mineralized bone area in the defect after 12 weeks in the bone graft group (i). TGF- β 3/PLA (ii) showed significantly more amount of calcium deposition over 12 weeks than PLA only (iii). Median values for the amount of mineralization after 12 weeks: (i) 45.1 %, (ii) 24.2 %, (iii) 5.18 %, (iv) 7.17 %.

Fluorescence microscopy showed at 4 and 6 weeks significantly more activity of mineralization in the

bone graft group (i) compared to TGF- β 3/PLA (ii) and PLA only (iii). The deposition of xylenol orange showed no significant differences between the groups at 8 and 10 weeks.

The qualitative analyses of the Giemsa/Eosin stained sections showed after 12 weeks the highest activity of bone formation in the bone graft group (i), where many round osteoblasts surrounded by wide ostoid seams were seen. Many osteoclasts and eroded surfaces as signs of resorption were mainly seen in the TGF- β 3/PLA group (ii). A bony bridging, crossing the defect was shown in 3 of 4 specimens of the bone graft group (i). Most vessels were seen in the TGF- β 3/PLA group (ii). Transverse connective tissue structures occluding the proximal and distal medullar cavities as signs of highest soft tissue in-growth were seen in the empty defects (iv). Most foreign body giant cells were found in the PLA only group (iii).

DISCUSSION & CONCLUSIONS: The study showed that TGF- β 3 enhanced bone formation, but the stimulation did not reach the potential of bone graft. Possible reasons could be an inaccurate dose or suboptimal release characteristics of TGF- β 3. Furthermore PLA did not show a sufficient potential for bone regeneration enhancement. Optimization in material type and structure of the carrier as well as in the release kinetics of the Growth Factor may provide improved results.

TGF- β 3 showed an osteoinductive potential. However effects of TGF- β 3 loaded on PLA were inferior to an autologous cancellous bone graft in an ovine segmental tibia defect after 12 weeks.

REFERENCES: ¹B.N. Summers and S.M. Eisenstein (1989) *J Bone Joint Surg. Br.* **71(4)**:677-80. ²P. Hardouin, K. Anselme, B. Flautre, et al (2000) *Joint Bone Spine* **67(5)**:419-24. ³P. ten Dijke, K.K. Iwata, C. Goddard, et al (1990) *Mol. Cell Biol.* **10(9)**:4473-79. ⁴Z. Gugala and S. Gogolewski (1998) *24th Annual Meeting of the Society for Biomaterials, San Diego, California, U.S.A.*:417.

ACKNOWLEDGEMENTS: Polymeric Implants: Sylwester Gogolewski; Statistics: Dominik Pfluger; Microscopy: Christoph Sprecher; Histology: Nora Goudsouzian.