In Vivo µCT-Analysis of BMP-2 Mediated Fracture Healing in Time Response in a Rabbit Radius Critical Size Defect (CSD) Model

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\textbf{Aims.} Pseudarthrosis is the movement of a bone at a defect site resulting from inadequate healing. Patients with non-unions usually feel pain at the fracture site long time after the initial pain of the fracture disappears. Treatment is usually made by bone graft which involves setting an additional bone defect at a second location accompanied by further painful processes. Approaches for enhancing fracture-healing can be broadly divided into two categories: mechanical or physical interventions, and biological interventions. One rationale for the use of biological agents to enhance fracture repair is that they are involved in both embryonal bone formation and fracture-healing. Thus, these factors have the potential to interact therapeutically in a process in which they are naturally involved\textsuperscript{(1)}. Among the factors identified in healing fractures, bone morphogenetic proteins (BMPs) have been implicated as key elements in the cascade of molecular events required for skeletal development and repair\textsuperscript{(2)}. The present study focuses on one member of this protein family: recombinant human bone morphogenetic protein-2 (rhBMP-2). In vitro, the primary effect of rhBMP-2 is to induce the differentiation of mesenchymal stem cells into cells expressing an osteoblastic phenotype. In vivo studies have shown that rhBMP-2 induces ectopic bone formation and promotes the healing of critical sized defects\textsuperscript{(3)} and spinal fusion sites\textsuperscript{(4)}. However, less is known about the ability of rhBMP-2 to promote the instauration of long bone, containing a bone marrow cavity and even less about its effects in the course of time in one individual. Therefore we investigated the formation of new built bone tissue focused on the variances in time in a not self healing rabbit radius CSD by in vivo µCT-analysis.

\textbf{Method.} Collagen sponges were coated with 20 or 50 µg of BMP-2 or left uncoated (control) and implanted into a not self healing rabbit radius CSD as described elsewhere\textsuperscript{(5)}. Anaesthetised animals were scanned in vivo directly postoperatively, 4, 8 and 12 weeks post surgery. For bone mineral density (BMD) scans the defect regions were resected from the euthanized animals and scanned in water tubes. Phantoms with predefined densities and CTAn-software were used for BMD-measurement as described in manufacturer's manual. For analyses three different Regions of Interest (ROIs) were configured – all of them in the defect centre – and compared to healthy long bone. The first included the total new built bone matrix (TV), the second was layered only over the cortical bone ring and the third included only the trabecular bone region excluding cortical bone. For morphological observation and quantification interpolated regions of interest were chosen, surrounding the ulna and the remaining radius endings. Then subtractive ROI function was used to analyse only the new built callus tissue. ROI shrink wrap function was used to analyse the total bone (tissue) volume (TV) including the not mineralised cavities, which are below the threshold for calcified bone volume (BV). This procedure was eminent as calcified matrix is reduced when callus tissue is reorganised to long bone, containing trabecular or marrow cavity structures, even the volume of the total bone is increasing.
Results. BMP-2 coated collagen sponges led to defect bridging in all examined animals 4 weeks post surgery. The major effect of BMP-2 was seen in the early tissue repair. At the 4 week time point BMP-2 received a 22fold increase of new built bone tissue compared to control. This difference abates over time. Thus - even the volume of new built bone is still increasing - the disparity to control halved to 11fold after 8 weeks of examination and declined further to 3.5fold at the 12 week time point (Fig.1). Morphological examination of the regenerative tissue showed massive callus tissue but no marrow cavity, both in the 20 µg and in the 50 µg BMP-2 group (Fig.5). Thus the proportion of calcified tissue to total bone volume is increased in the BMP-2 mediated repair tissue compared to healthy bone (Fig.2). No dose dependant effect was obvious, only a trend to less bone in the 50 µg treated animals was seen (Fig.3). Bone mineral density did not change for cortical ring and total bone analysis but was increased 3.13fold in the trabecular bone region by BMP-2 compared to healthy bone (Fig.4).

Fig.1: Time response of total new built bone volume (TV) after treatment with 50µg of BMP-2 compared to the untreated control defect; Fig.2: Proportion of calcified fraction (BV) to total bone volume (TV) after treatment with 20 or 50 µg BMP-2 compared to healthy bone and untreated control defects 4 weeks post surgery; Fig.3: Total Bone volume (TV) 4 weeks after treatment with 20 or 50 µg of BMP-2; Fig.4: BMD in BMP-2 treated defects 12
weeks post surgery in ex-vivo probes compared to healthy bone. Fig.5: Cross and longitudinal section 8 weeks post surgery of controls (C) and BMP-2 group (B)

Conclusion. μCT-analyses allowed morphological examination and quantification of fracture repair tissue in a rabbit radius defect model in time response in one and the same defect. Thereby animal numbers needed were drastically reduced and development over time could be investigated in one individual - eliminating interindividual variances between time points. We could approve the bridging of a not self healing long bone defect by BMP-2 already 4 weeks post surgery and conclude that early fracture healing is arbitrative for joining of non-unions. However, BMP-2 treatment appears adequate for reconnecting not healing fractures, but did not lead to functional instauration of long bone containing a bone marrow cavity - potentially due to an insufficient angiogenic impact\(^{(5,6)}\) – what remains to be investigated in further studies.

References