

OSTEOGENIC ACTIVITY OF OP-1 BONE, MORPHOGENETIC PROTEIN-7 (BMP-7), IN A HUMAN FIBULAR DEFECT MODEL

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Introduction Bone is among the most frequently transplanted tissues in the human body. Although either allografts or autografts can be used as bone transplants each of these tissues has lots of disadvantages; autologous bone is the most effective osseous material but serious disadvantages include morbidity at the donor site and a limited availability. Differently prepared allografts are easily available and appropriate to fill larger bone defects, but infer risks related to immunological reactions, transmission of diseases or loss of biological potential. Therefore a synthetic material avoiding these problems and offering osteogenic, osteo-conductive and osteo-inductive properties comparable to autologous bone tissue, could help healing bone defects of different sizes. OP-1 protein (BMP-7) in a collagen matrix has osteogenic, osteoconductive and osteoinductive properties and could therefore potentially be used heal bone defects. When implanted in animals, various preparations of the OP-1 device caused new bone formation in intra skeletal sites. The OP-1 device is formulated with a bovine bone Type I collagen matrix. However unto now there are no clinical prospective studies in humans comparing the bone inductive properties of BMP's with more frequently used bone inductive materials such as demineralized bone. This prospective study was designed to investigate the effectiveness of OP-1 on a collagen Type-I carrier in a critical sized human fibula defect. This was tested in patients undergoing tibial osteotomy (because of osteoarthritis) in which the commonly performed fibular osteotomy was used as the study model.

Materials and Methods This study consisted of two phases, each including two treatment modalities in 26 patients undergoing tibial osteotomy because of osteoarthritis. The high tibial osteotomy was fixed by staples. The fibular osteotomy was performed at the junction of the middle and the distal one third of the fibula via a separate skin incision. An osteo-periosteal segment was removed. The fibular defect was created in such a way that after the osteotomy of the tibia a defect measuring approximately 15 mm was left. After surgery a splint bandage was applied in 10 degrees of flexion of the knee. Post-operative management included six weeks circular plaster cast from one week post-operatively. Protected weight bearing was allowed from two weeks on.

The first phase concerned the validation of the fibular osteotomy as a study model, using positive, demineralized bone (DMB), and negative (sham treatment) controls. The negative control was left untreated. The DMB consisted of 2 ml demineralized dust with glycerol (50% by its volume) resulting in an osseous gel (Grafton, BIS, Leiden, The Netherlands).

The second phase concerned the osteogenic potential of OP-1 on collagen Type-I versus collagen Type-I alone. OP-1 (Novos, Stryker Biotech, Natick, MA, USA) consisted of 2.5 mg recombinant human osteogenic protein-1 and 1 g purified insoluble bovine bone Type-1 collagen, reconstituted with 3 cc 0.9% NaCl. Collagen alone was equal to the Type-1 carrier of OP-1.

Blinding procedure: All treatments were blinded for the patients. In addition OP-1 and the carrier were also blinded for the medical staff and investigator.

Evaluation: The patients were evaluated clinically, radiographically and by Dual Energy X-ray Absorptiometry (DEXA) preoperative and postoperative at one week, 6 weeks, 10 weeks and 4, 6 and 12 months follow up. Clinical evaluation consisted of determining the Hospital for Special Surgery knee score, assessment of pain and appraisal of patient satisfaction. Radiographs were evaluated for changes in the fibula defect by two orthopaedic surgeons independently. Finally DEXA was used to measure quantitative changes over time in bone mineral density (bmd) within the defect, by two independent and blinded observers.

Statistics: The pre-operative bmd values (baseline) varied among the treatment groups. To prevent additional intergroup variability to further follow-up, one way analysis of covariance's (ANCOVA) was used to estimate bmd differences between the treatment groups, adjusted for the preoperative bmd variability between the patients. Before starting the bmd

values were first transformed to logarithmic values to achieve more equality of variances. Mean bmd differences between the treatment groups, adjusted for the pre-operative variability between the patients, were calculated at each follow-up period. A p-value of less than 0.05 was assumed to establish a significant difference. The data were analyzed with SPSSWIN 7.5 PC computer software.

Results Twenty-four patients completed this investigation, 6 patients in each treatment group. The clinical results (HSS-score, pain assessment, and patient satisfaction) were comparable among the treatment groups, except for pain complaints at the fibular site, which were higher in the OP-1 device group. The radiological and DEXA results of the **first phase** of this study prove the critical size of the defect. In the sham treatment group hardly any bone changes were observed while, in the demineralized bone group, new bone formation was visible by 6 weeks follow-up and bridging in 4 of 6 patients by 1 year.

The results of the **second phase** of this study established that no significant new bone formation occurred in the presence of collagen alone. In the OP-1 group, all patients except 1 demonstrated new bone formation by 6 weeks and bridging by 1 year. The DEXA results of the OP-1 and the demineralized bone group showed that the bmd of patients treated with OP-1 did not differ from those treated with DMB. Also the results of the patients treated with collagen or patients who were in the sham treated group were not statistically different. Patients treated with OP-1 had significantly higher bmd values of the total defect than patients treated with collagen alone from 4 months (p=0.038) on. Radiologically the pattern of bone reactions followed by bridging were different between the OP-1 treated and the DMB-treated patients. While the DMB patients showed a more centrally healing pattern, starting at the corners of the fibula, in the OP-1 patients bone formation started medially or laterally at the external borders of the defect. The subjective bone bridging seemed to occur slightly earlier in the OP-1 treated group; the patients in the DMB group appeared to have more dense bone over time.

Immunogenicity results: Clinically none of the patients showed a significant reaction at the operation site. The Elisa tests showed that two patients could be listed as having a significant anti-collagen reaction, but this did not affect the healing of the fibula defect.

Discussion In this study the osteogenic activity of the OP-1 device is compared to a sham, the OP-1 carrier alone and demineralized bone. In the **first part** of the study the fibula model was validated by comparing the results obtained in the Sham and the DMB group respectively. While in the Sham treated group hardly any bone formation was seen on X-ray or measured using DEXA, the DMB treated group showed that the defect is easily healable if treated with a standard allograft procedure. Therefore this model used a **critical size defect** which will not heal if left untreated. The **second part** of the study, a prospective double-blind randomized trial between the OP-1 device and the collagen carrier as a control is therefore allowed to be evaluated in this study model. The results indicate that the patients treated with the carrier alone show only minimal signs of bone formation, never leading to bridging of the defect. Therefore collagen alone does not have the property to stimulate precursors of bone formation to form bone. It was suppose, as was suggested by other authors, that due to cross-linking of the fibers, only little potential for bone repair is left. In contrast the OP-1 group showed extensive bone formation; already at 6 weeks 4 cases had bridged fibula defects. The bmd-curves showed increasing mineralization over time, with restoration of the pre-operative baseline values at one year after follow-up.

Conclusion: The results of this study proved that the fibula defect model is a very good critical size defect model for the quantification of bone formation. Although the patterns of bone formation between DMB and OP-1 are quite different, the osteogenic activity of OP-1 in a human critical size defect in this prospective double blind trial is established with certainty.

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