OSTEOGENIC GROWTH PEPTIDE MODULATES FRACTURE CALLUS STRUCTURAL AND MECHANICAL PROPERTIES

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Introduction: The osteogenic growth peptide (OGP) is a naturally occurring tetradecapeptide identical to the C-terminal amino acid sequence 89-102 of histone H4 (H4) and it is a key factor in the mechanism of the systemic osteogenic response to local bone marrow injury. It has been recently shown that OGP increases the dimensions of fracture callus and modulates the expression of bone anabolic regulators. It also accelerates cartilage-to-bone transition (Brager et al. 2000, Sun and Asshurst 1999). The present study was designed to test the hypothesis that as in the case of the structural parameters, OGP modulates the biomechanical properties of the callus. Indeed, using a combined biomechanical, micro-computed tomographic (microCT) and histomorphometric approach, we demonstrate an OGP-induced, marked enhancement of the fracture callus (initial stage of healing) toughness and its relationship to the connectivity of the mineralized callus.

Methods: The experimental protocol was approved by the Institutional Animal Care and Use Committee, Faculty of Medicine, the Hebrew University of Jerusalem, Israel. Closed, unilateral mid-diaphyseal femoral, unstable fractures were performed in male Sabra rats weighing 290 g. Pre-fracture fixation was accomplished by retrograde insertion of an intramedullary pin through a median parapatellar incision at the knee. Standardization of fracture configuration was determined radiographically. OGP (25 ng/rat/day) or the phosphate-buffered saline (PBS) vehicle alone were administered systemically by subcutaneous injections commencing the first post-operative day (6 days/week) and the fractured bones were harvested 1, 2, 3 and 4 weeks post-operatively. Thirteen fractured femurs from each time/treatment group were subjected to microCT morphometric evaluation followed by biomechanical torsional testing. Additional 4-6 specimens per time/treatment group were studied by histomorphometry. The primary statistical analysis of differences in quantitative microCT, biomechanical and histomorphometric parameters between time/treatment groups was carried out using analysis of variance (ANOVA). When significant differences were indicated by ANOVA, group means were compared using the Tukey test for pairwise comparisons

Results: Jointly, the quantitative microCT and histomorphometric analyses of the callus showed an OGP-induced anabolic shift in the callus remodeling which after four weeks maintained the total callus and bone volumes at the 3-week level; control animals at four weeks showed significantly lower values for these parameters (figure 1, A and B). Accordingly, the 4-week total connectivity in the OGP-treated fractures was 46% higher compared to controls (figure 1, C). Three-dimensional imaging of the callus revealed that direct bridging between the fracture ends occurred only in the OGP-treated fractures (figure 2). Torsional testing of the same specimens that were before analyzed by microCT showed a greater fracture toughness consequent to the OGP administration (~62% and ~29% higher than control after three and four weeks, respectively) (figure 1, D). The highest biomechanical-microCT correlation was found between the toughness and the connectivity density (table 1).

Discussion: These results confirm previously published observations on the OGP-induced acceleration of cartilage-to-bone transition in fracture callus and highlight the three-dimensional increase in total volume and connectivity. Moreover, the OGP-treated group shows a higher callus resistance to re-fracturing. In addition, these results provide the first indication for a functional relationship between a structural parameter (connectivity) and a major biomechanical property (fracture toughness). Collectively, the present and previous experimental promotion of fracture healing by systemically administered OGP suggest this peptide and/or its derivatives as a potential therapy for the acceleration of bone regeneration in instances of fracture repair and perhaps other bone injuries.

Abbreviations and comments: BV_l, total volume of low radio-opacity bone (represents mainly newly formed bone); BV_h, total volume of high radio-opacity bone (represents pre-fracture cortical and newly formed mature bone); BV_t, combined volume of BV_l and BV_h; Conn.D, connectivity density.

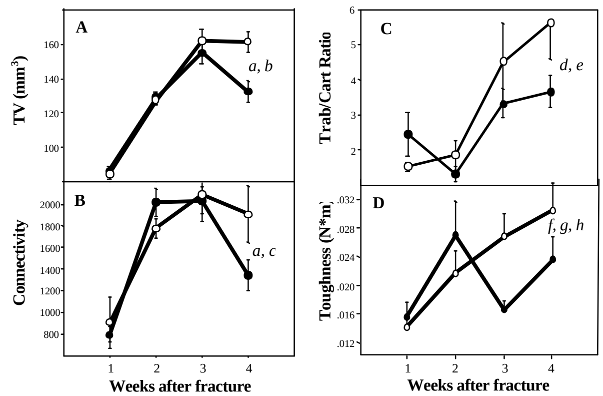

Figure 1. MicroCT, histomorphometric and biomechanical properties of the fracture callus. OGP group o ; Control group • ; Quantitative microCT measurements of the mineralized callus (A, total volume [TV]) and connectivity (B). Mean±SE; *a*, $p< 0.001$ for temporal trend; *b*, $p= 0.021$ and c, $p= 0.037$ for effect of OGP over controls. C, the histomorphometric assessment of the bone/cartilage ratio shows that OGP stimulates cartilaginous-to-osseous callus transition following rat femoral fractures. Mean±SE; *d*, $p< 0.001$ for temporal trend in week 2, 3 and 4 time groups; *e*, $p= 0.024$ for effect of OGP over controls in week 2, 3 and 4 time groups. D, OGP enhances toughness of early fracture callus in rat femur. Mean±SE; ; *f*, $p= 0.005$ for temporal trend in OGP group; *g*, non-significant temporal trend in PBS group; *h*, $p= 0.01$ for interaction between treatment and time after fracture in week 3 and 4 time groups.

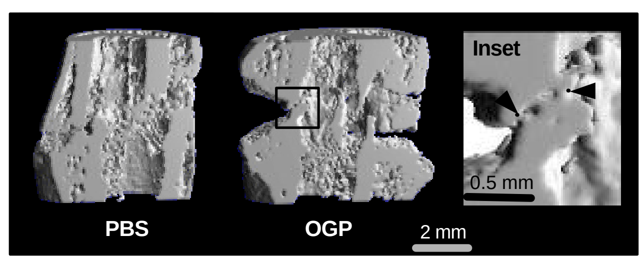

Figure 2. 3-D microCT images of fractured femora from representative specimens with median TV values, 4 weeks after fracture. Inset, high magnification of framed zone in OGP-treated group. Note partially remodeled cortical union (black arrows).

Table 1. Correlation coefficients between fracture callus toughness and structural microCT total parameters

Parameter	BV_l	BV_t	BV_h	TV	Conn.D
Toughness to torsion	0.363 p<0.001	0.341 p<0.001	0.082 N.S.	0.342 p<0.001	0.632 p<0.001
BV_l		0.979 p<0.001	0.780 p<0.001	0.959 p<0.001	0.714 p<0.001
BV_t			0.584 p<0.001	0.941 p<0.001	0.632 p<0.001
BV_h				0.837 p<0.001	0.004 N.S.
TV					0.778 p<0.001

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