INTRODUCTION

The auto-inductive capacity of bone is the basis of the numerous grafting procedures performed in orthopedic surgery. However, the increasing number of grafting procedures and the disadvantages associated with graft harvesting drive the quest for alternative methods to regenerate bone tissue. A promising strategy that may obviate the need for bone grafts is the use of bioactive molecules that regulate the auto-induction process during bone regeneration.

Bone morphogenetic proteins are potent osteoinductive growth factors which play a central role in most bone regeneration strategies for localized defects. Parathyroid hormone (PTH) is one of the major systemic regulators of bone metabolism. The anabolic features of intermittent PTH administration have made it particularly appealing for the treatment of osteoporosis, in which it was shown to increase the bone mass and reduce the fracture rate. Given the enhancement of bone formation by the local application of BMPs and the systemic application of PTH, combination therapies may be beneficial for bone tissue engineering. The aim of this study was to investigate the effect of PTH, BMP-2 and combination treatments on the ectopic and orthotopic bone formation.

METHODS

Experimental design: A total of 54 rats were used for the experiment, for which the Institutional Animal Care and Use Committee gave approval. Each rat received an unloaded or BMP-2-loaded scaffold subcutaneously in the left leg and in a 5 mm defect in the right femur. Femur defect implants without implants served as controls in the orthotopic site. Each of the surgical treatments was studied alone or in combination with PTH injections administered subcutaneously in the neck daily. The implants were removed after 8 weeks of implantation for the evaluation of bone formation by micro-computed tomography (CT), dual energy X-ray absorptiometry (DEXA) and histology.

Implants: The composite consisted of unloaded or BMP-2 loaded poly(lactic-co-glycolic acid) (PLGA) microspheres incorporated into a solid poly(propylene fumarate) (PPF) rod which was surrounded by a cylindrical gelatin hydrogel.

DEXA analyses: The bone mineral density (BMD) of the subcutaneous and femoral defect implants was measured by DEXA using a PIXImus densitometer. To investigate the effect of PTH on existing bone, the BMD of the humerus and L1 vertebra were also analyzed by DEXA.

μCT analysis: The implants were scanned on a custom-built μCT system and the projections were reconstructed into a 3-dimensional image with a 20 µm resolution. Image analysis was then performed using the Analyze™ software package. The reconstructions and volume quantification of the ectopic and orthotopic bone were obtained using standardized thresholds.

Histology: The quality of the newly formed bone was studied by histology. After the μCT analysis, the implants were dehydrated in series of alcohol, embedded in methylmethacrylate, sectioned and stained with Goldner’s trichrome or methylene blue/basic fuchsine for histology.

Statistical analysis: All data are given as means ± standard deviations for n=9. Comparison of ectopic BMD and bone volume was performed by an independent t-test. Outcomes for the orthotopic implantations were compared using ANOVA with Bonferroni’s post hoc tests. The level of significance was set at p <0.05.

RESULTS

Analysis of the systemic PTH effect on the existing bone showed that intermittent PTH(1-34) administration resulted in a significant increase of the BMD of the right humerus and vertebra L1. At the ectopic site, PTH administration did not result in bone formation, whereas local release of BMP-2 significantly enhanced bone formation (Fig.1). PTH administration significantly increased BMD and bone volume in the ectopic BMP-2 implants (p<0.02). Histology showed that the newly formed bone had a woven or lamellar appearance.

DISCUSSION:

This study clearly shows that BMP-2-induced osteogenesis can be enhanced by intermittent administration of PTH. Although an anabolic effect on the rest of the skeleton was seen, PTH treatment had no significant effect on local bone regeneration in the unfilled and empty scaffold-filled defects. Implantation of BMP-2-loaded scaffolds resulted in a significant increase of bone mineral density and bone volume in both sites as opposed to the empty scaffolds. Although PTH alone did not significantly improve bone formation, it can enhance for local bone regeneration in combination with BMP-2. Based on these results, the PTH/BMP-2 combination therapy could be considered for the restoration of large bone defects in orthopedic surgery.

ACKNOWLEDGEMENTS: This work was supported by Mayo Foundation, NIH (R01 AR45871 and R01 EB003060), and the Netherlands Foundation for Health Research and Development ZonMW (Agiko 920-03-325).

Paper No. 177  •  55th Annual Meeting of the Orthopaedic Research Society

All orthotopic sites showed some newly formed bone at the defect edges (Fig. 3A). Almost full cortical regeneration was seen in all of the defects containing the BMP-2 loaded implants. The defect filled with BMP-2 implant contained significantly more bone compared to the other groups (p<0.04). The combination of BMP-2 and PTH resulted in a significantly higher BMD and bone volume compared to BMP-2 alone (p<0.04). No significant difference was found between the untreated and PTH-treated unfilled or empty scaffold filled defects.