Deleterious Effects of Intermittent Recombinant PTH on Cartilage Formation in a Rabbit Microfracture Model

INTRODUCTION

Endogenous parathyroid hormone (PTH) is the primary regulator of calcium and phosphate homeostasis. In times of low calcium, PTH stimulates osteoclastic resorption of bone, liberating calcium from the skeleton. Exogenous administration of intermittent PTH has considerably different effects. It has been shown in pre-clinical and clinical models to have potent anabolic effects on bone remodeling. Numerous pre-clinical studies have demonstrated that intermittent, low dose PTH administration can enhance fracture healing in an animal model. Holzer, et al. found increased callous formation and fracture healing in a rat model. Further studies found that systemic administration of PTH enhanced fracture healing by increasing bone mineral content, density, and strength. Similar results have been found in multiple studies using spinal fusion as a model as well.

The mechanism of enhanced fracture healing via intermittent PTH has been studied as well. Nakajima, et al. found increased levels of type I collagen, osteonectin, and osteocalcin in a fracture model treated with intermittent PTH compared to controls. In addition, intermittent PTH administration has been found to increase chondrocyte recruitment and maturation early during fracture healing as well as an increase in Wnt signaling. Despite the success of exogenous PTH on fracture healing and spine fusion, there have been few studies that have examined the role of PTH on cartilage formation. Kudo, et al. found that continuous PTH administration inhibited the healing of full thickness chondral defects in a rabbit microfracture model. The authors found that application of intermittent PTH compared to controls. In addition, intermittent PTH administration has been found to increase chondrocyte recruitment and maturation early during fracture healing as well as an increase in Wnt signaling.

The purpose of this study was to determine the effects of short term administration of intermittent PTH on cartilage formation in a well described rabbit microfracture model. We hypothesized that, based on the fact that intermittent PTH administration enhances fracture healing via increased chondrogenesis in a fracture healing model, it would increase the cartilage formation in a rabbit microfracture model.

METHODS

Study Design: The study was approved by the institute’s animal review board prior to initiation of the study. Twelve female New Zealand White (NZW) rabbits were used in the study. Animals were divided into three treatment groups: (1) Microfracture alone; (2) Microfracture and administration of 10 μg/kg PTH daily for 7 days; and (3) Microfracture and administration of 10 μg/kg PTH daily for 28 days. Non-operated contralateral knees were used as a control. The PTH was administered daily via subcutaneous injection commencing on the day of surgery and continued until the indicated time. Animals were sacrificed 90 days after the surgical procedure with an overdose of sodium pentobarbital. Radiographs were obtained at the time of sacrifice.

Surgical procedure: The rabbits were anesthetized with sodium pentobarbital. The skin overlying the left knee was prepped and a midline incision was made. A medial parapatellar arthrotomy was performed and the knee was flexed and the patella was subluxed laterally to expose the trochlea. Full thickness defects with the depth of 4 mm and a width of 6 mm were created in the weightbearing portion of the trochlea. The animals were administered pain medicine as needed and allowed to ambulate immediately after the procedure.

Histologic Analysis: At the time of sacrifice, the distal femur was isolated from the surrounding tissue and fixed in 4% paraformaldehyde at room temperature for 24 hours, and then decalcified with 10% ethylenediaminetetraacetic acid for 2 weeks. The specimen was embedded into paraffin for histological analysis. Each specimen was stained with hemotoxylin and eosin as well as alcian blue. The histologic specimens were scored as described by Pineda, et al. with a minimum of 0 and maximum of 14.

Statistical Analysis: The gross and histologic specimens were examined in a blinded manner and statistical significance was determined using an ANOVA or Mann-Whitney U test. A p value of <0.05 was considered statistically significant.

RESULTS

All 12 animals survived the entire study period. One animal in the microfracture alone group had considerable calcification on radiographs, but no other radiographic abnormalities were noted. On gross inspection, animals in the microfracture alone group demonstrated complete fill of the chondral defect with normal appearing cartilage. In the microfracture+1 week PTH group, there was fair to poor fill of the chondral defect with soft cartilage formation. In the microfracture+4 week PTH group, there was moderate fill of the lesion although the cartilage did not appear to be normal. Histologically, the cartilage in the microfracture alone group closely resembled normal cartilage although there were fewer chondrocytes with less architecture than the control animals (Figure 1). In both PTH treatment groups, there was minimal cartilage formation within the defect. The average histologic score in the microfracture alone group was 10.3 (Range 9-13), compared to 2.7 (Range 1-6) in the microfracture+1 week PTH and 3.4 (Range: 1-8) in the microfracture+4 week PTH group. These differences were statistically significant compared to the microfracture alone group (p<0.001 vs. microfracture alone).

DISCUSSION

Full thickness cartilage lesions remain a difficult problem for the orthopaedic surgeon. There is increasing evidence that mesenchymal stem cells that migrate into the lesion following microfracture can be stimulated to enhance chondrogenic differentiation and maturation. Despite the success of intermittent exogenous PTH in the treatment of fracture healing by enhancing chondrogenic maturation, this study found that intermittent PTH had a deleterious effect on the healing of a full thickness chondral lesion. Interestingly, both short and long term dosing of PTH had a similar effect on inhibiting cartilage formation in this model, similar to previous data that demonstrated inhibition of cartilage formation in a continuous PTH administration model.

Although discouraging from a cartilage repair point of view, our data is consistent with previous studies using continuous PTH in a cartilage repair model. Other studies have demonstrated prolonged exposure to exogenous PTH can result in morphological changes in the cartilage and breakdown of the extracellular matrix in the chondroprogenitor zone. Furthermore, this data is important as it suggests that the role PTH administration has in clinical fracture healing must be examined carefully. Although PTH is likely very beneficial to promote healing in spine fusion and in midshaft fractures, its deleterious effects on cartilage in this study suggests that it may have adverse effects on the outcomes of fractures such as tibial plateau injuries that require cartilage healing for a successful clinical outcome.

REFERENCES