The Effect of a Vitamin D Deficiency on Bone and Cartilage in the Meniscectomized Guinea Pig Model of Osteoarthritis

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Introduction: Articular cartilage is required for normal joint function. Osteoarthritis (OA) is a common joint disease characterized by the degeneration of articular cartilage as well as all tissues of the joint. Bone changes are important determinants for OA progression(1) and Vitamin D is known to promote bone health(2). The Framingham study showed that a low intake and low serum levels of vitamin D appear to be associated with an increased risk of OA progression in the knee(3). Low serum levels of vitamin D are associated with incident changes of radiographic hip OA characterized by joint space narrowing(4). The male Dunkin-Hartley guinea pig spontaneously develops OA. A medial meniscectomy creates an accelerated model of this disease. In this study we wish to determine if a vitamin D deficiency accelerates the OA process.

Materials and Methods: Male Dunkin-Hartley guinea pigs were used to investigate the effects of vitamin D deficiency on bone and cartilage in OA. The animals were received at 2 months of age, and vitamin D modulation began upon arrival. There were two treatment groups: vitamin D deficient and control diet group with 8 animals per group. The vitamin D deficient group was fed a special diet formulated with no vitamin D and the control diet group was fed a normal guinea pig diet containing 153 IU of vitamin D/day. After one month of acclimatization, a left medial meniscectomy (MNX) was performed and the animals were sacrificed 1, 3 and 6 months post MNX. Serum Analysis: 25(OH)D blood serum levels were analyzed using a DiaSorin radioimmunoassay. ALP, Ca, P serum metabolites were investigated. Calciﬁcation: Renal calcification was determined using wet ashing. Cartilage: Representative sections of the tibial plateau were stained with Safranin-O and a fast green counter stain and cartilage changes were graded using a modiﬁed Mankin scale. The progression of the tidemark and osteophyte area were evaluated on the Safranin-O stained sections. Bone The BMD of the excised femora was analyzed by DXA. The distal femur and proximal tibia were imbedded in Spurr and stained with Goldner’s trichrome. Bioquant Bone Morphometry software was used to determine bone histomorphometric properties which comply with ASBMR guidelines. Mineralization proﬁles of the tibia were determined using quantitative backscattered electron imaging on the subchondral trabecular bone. SEM images were also used analyze the connectivity of the subchondral trabecular bone. The subchondral bone thickness was measured using SEM images. Finally, the blocks previously used for SEM imaging were used for microhardness.

Statistical Tests: ANOVA was used to determine if there were differences across the time points. A t-test was used to evaluate the difference between the mean of the groups at each time-point. A p-value ≤ 0.05 was taken as a significant difference. Data is reported as mean ± standard deviation.

Results: The vitamin D dosing caused the expected 25(OH)D serum response. There was a signiﬁcant (p≤0.05) increase in 25(OH)D serum level for the control diet compared to the vitamin D deﬁcient diet. No diﬀerences were seen in renal calcification. Ca homeostasis was maintained. A decrease (p≤0.05) in serum P was seen in the vitamin D deﬁcient group, while a increase in ALP was seen in the no vitamin D group. By 6 months an increase (p≤0.05) in the Mankin score was seen for the vitamin D deficient animals compared to those on the control diet. The tidemark in the vitamin D deﬁcient animals progressed closer to the surface of the articular cartilage (p≤0.05), while the subchondral bone plate thickness increased (p≤0.05) with time. The BMD for the vitamin D deﬁcient group was lower (p≤0.05) compared to the control diet 6 months post meniscectomy. The structural histomorphometric parameters, seen in Table I showed an increase (p≤0.05) in bone volume from 1 to 3 months post meniscectomy, this increase in bone volume was maintained in the control diet group, while a decrease (p≤0.05) in bone volume was seen for the vitamin D deﬁcient group from 3 to 6 months post meniscectomy. No changes in osteoid were seen over time for the vitamin D deﬁcient group.

Discussion: Dosing was effective at increasing the serum 25(OH)D levels and the calcium homeostasis was maintained. The drop in phosphate is likely due to an increase in parathyroid hormone and loss of phosphate into the urine. The increased ALP may reﬂect a mineralization defect in the vitamin D deﬁcient animals. The progressing tidemark suggests a decrease in cartilage thickness and increase in calcified cartilage. Vitamin D deﬁciency seems to be having a strong eﬀect on bone quality. A vitamin D deﬁciency may have caused an increase in PTH to maintain calcium homeostasis by stimulating osteoclastic bone resorption, which may have impaired bone volume and connectivity in this group. The deteriorated bone quality seen in the vitamin D deﬁcient group may be instrumental in cartilage changes. The decrease in vitamin D may impair the ability for the bone to adapt to changes in the load which may have contributed to the cartilage degeneration. It has also been reported that unmineralized bone creates a deforming, erosive form of OA. The impaired mineralization, microhardness and bone quality of the vitamin D deﬁcient bone may predispose the joint to further OA progression.

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