IMMUNOHISTOCHEMICAL LOCALIZATION OF OSTEOGENIC PROTEIN-1 AND ITS RECEPTORS IN RABBIT ARTICULAR CARTILAGE

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Introduction: Bone morphogenetic proteins (BMPs), initially isolated from bone matrix, comprise a family of proteins involved in the growth and development of a variety of cells and tissues. Members of this family have been found to be particularly important in bone and cartilage differentiation, growth and remodeling (1). Among these BMPs is osteogenic protein-1 (OP-1) which has also been shown to be endogenously expressed (both message and protein) in human articular cartilages (2). Furthermore, OP-1 is present in human adult articular cartilage in two forms, inactive (pro-) and active (mature) (2). OP-1, like other BMPs, binds and initiates a signaling cascade via a complex of type I and type II BMP receptors. BMP receptors exist in two types: type I (ALK-1-6) and type II (3). OP-1 has been shown to transduce the signal through type II BMP receptor and Type I BMP receptors (ALK-2, 3 and 6) (4). The purpose of our study was to investigate whether endogenous OP-1 and the representatives of its signaling pathway could be detected in normal intact rabbit articular cartilage, and in a model of enzymatically induced cartilage damage. In addition, the relationship between the endogenous bone changes and cartilage degeneration in both human and experimentally induced osteoarthritis has yet to be elucidated. We, therefore, also used the bisphosphonate, zoledronate to observe the effect of the inhibition of bone remodeling on the presence of endogenous OP-1 and its receptors in both the bone and the overlying articular cartilage. In the present study we compare the localization and distribution of both forms of OP-1 (pro- and mature) and its receptors, type I and type II (ALK-2 and ALK-3).

Methods: Adolescent New Zealand white rabbits were assigned to one of three groups: untreated (3 rabbits); injection of 2 mg chymopapain (CP) into the left knee joint (4 rabbits); or thrice weekly subcutaneous injections of 10 µg zoledronate/kg body weight (4 rabbits), a dosage shown to be effective in the inhibition of bone remodeling in the rabbit (unpublished data). After sacrifice (56 days post CP injection), whole patellae from both knee joints were decalcified, paraffin embedded, sectioned to 6 µm thickness and stained with Safranin O (6). Immunohistochemistry was performed with five types of antibodies (provided by Stryker Biotech and Ludwig Cancer Institute): anti-pro-OP-1 polyclonal antibody, anti-mature OP-1 monoclonal antibody, anti-type II BMP receptor polyclonal antibody, and two polyclonal antibodies to type I BMP receptor (ALK-2 and ALK-3). For negative controls, the primary antibodies were replaced with either: a) normal serum, b) secondary antibody alone, or c) primary antibody preabsorbed with OP-1. The ImmunoPure ABC Immunohistochemistry Kit (Pierce) was utilized with biotinylated rabbit anti-goat IgG or horse anti-mouse IgG as secondary antibodies. To inhibit endogenous alkaline phosphatase activity, ImmunoPure Phosphatase Suppressor (Levamisole) was used.

Results: Safranin O staining substantiated that CP injection resulted in cartilage matrix damage as expressed by fribillation of the surface, and depletion of proteoglycans and some chondrocytes. All other patellae, including the contralateral patellae of CP injected joints displayed no damage of articular surfaces with strong proteoglycan staining throughout the tissue. By immunohistochemistry both forms of OP-1 (pro- and mature) were identified in chondrocytes and osteocytes of rabbits from all groups. However, there were detectable differences in the intensity of staining and cartilage layer distribution of pro- and mature OP-1. Pro-OP-1 was detected in the superficial, middle and deep zones of cartilages from all groups. Osteocytes were also positive for pro-OP-1 throughout the patellae in all groups of rabbits. There was no detectable pro-OP-1 in calcified cartilage, cartilage matrix or bone matrix. Strong staining for mature OP-1 was detected in the middle and deep zones of articular cartilage of both joints from untreated and zoledronate treated groups. However, in contrast to pro-OP-1, there was little or no positive anti-mature OP-1 immunostaining in the superficial zone. Within the CP treated rabbits, the regional distribution of anti-mature OP-1 stain was identical to that of the untreated and zoledronate treated groups. However, staining was more intense in the patellae of the CP injected knee than in the un.injected knee and more intense than that of the untreated and zoledronate treated groups. In all experimental groups mature OP-1 was also detected in the osteocytes throughout the bone, while no staining was found in the cartilage and bone matrix or calcified cartilage.

Concerning OP-1 receptors, type II BMP receptor and type 1 BMP receptor (ALK-3) were found with moderate to strong levels in chondrocytes of the superficial, middle and deep cartilage zones and in osteocytes throughout the bone in all groups. Staining for these receptors was never detected in the calcified cartilage zone. Anti-ALK-2 stain was either absent or appeared in the superficial, middle and deep articular cartilage zones with less intensity than anti-ALK-3 or anti-type II receptor staining. The presence of BMP receptors was similar in all treatment groups. No matrix staining was detected with any of the receptor antibodies.

Discussion: The present study was a continuation of ongoing investigations on the understanding of the biology of OP-1 and its receptors in articular cartilage under physiological and pathophysiological conditions. Two animal models were utilized: 1) the CP-induced cartilage damage model previously shown to mimic the cartilage matrix pathology seen in early stages of osteoarthritis (7); and 2) a model of inhibition of bone remodeling. We chose the first model to observe changes in endogenous OP-1 and its receptors after catabolic injury. We have found that experimentally induced matrix destruction (CP-treatment) increases the detectable levels of mature OP-1 present in the tissue, possibly indicating a reparative response by the chondrocytes. The model based on the inhibition of bone remodeling was chosen to study the influence of bone changes on OP-1 protein and its receptors in the overlying articular cartilage. A relationship between subchondral bone changes and articular cartilage degeneration in osteoarthritis is well known, however, the mechanism of the interaction between these two tissues has yet to be elucidated. In the present study, we have found that the inhibition of bone remodeling by the bisphosphonate, zoledronate, had no effect on cartilage OP-1 and its receptors. In conclusion, we have demonstrated that endogenous OP-1 could be detected in adolescent rabbit articular cartilage. Interestingly, as in human cartilage, both forms of OP-1 (pro- and mature) are found in rabbit cartilage. However, the distribution of these forms is different from those described in humans as well as different between experimental groups. In rabbits, while pro-OP-1 was present in all cartilage layers, mature OP-1 was detectable in the middle and deep, but only weakly or not detectable in the superficial layer, which indicates, perhaps, a distinct processing of OP-1 by superficial layer chondrocytes in rabbits. The present study shows that OP-1 and its receptors have been identified in rabbit articular cartilage and bone suggesting a possible role for this pathway in cartilage and bone homeostasis.


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