Bone sialoprotein distribution in guinea pig osteoarthritis

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Morphological studies of osteoarthritis (OA) have largely been performed on surgical specimens from patients with advanced disease. Such specimens do not allow conclusions on the early events of OA and consequently little is known about initiating agents and pathogenetic mechanisms. To overcome these obstacles, various animal models have been designed. Most of these models, however, involve an intraarticular intervention resulting in rapidly progressive changes, quite unlike primary osteoarthrosis. There are, however, naturally occurring OA-like conditions in animals, e.g. Dunkin Hartley guinea pigs. In a recent study we have presented quantitative data on articular cartilage and subchondral bone during development of guinea pig OA. Previous studies have shown that bone changes are important in the OA pathogenesis (Radin 1986).

Osteoblasts produce substances of low molecular weight, which may regulate matrix assembly and mineralization. One of them, bone sialoprotein (BSP) binds tightly to hydroxylapatite and is mainly found in mineralized tissues. BSP is concentrated to the border between mineralized articular cartilage and subchondral bone.

The objective in this study was to investigate the BSP distribution with ultrastructural immunohistochemistry in guinea pig OA.

Materials and methods

Male Dunkin Hartley guinea pigs 12 months old were heparinized, anaesthetized and fixed by vascular perfusion using 0.1M phosphate-buffered fixative of 0.3% glutaraldehyde and 0.3% paraformaldehyde, pH 7.4, containing 3% dextran T40. Subsequently the proximal tibiae were dissected out, thin vertical slices from the central medial and lateral plateaus including cartilage and subchondral bone were cut into small pieces. The specimens were dehydrated in methanol and embedded at low temperature in the polar resin Lowicryl K11M. Two ultrathin sections (35–40 nm) were cut from two independent blocks from each animal. The specimens were placed on formvar-coated nickel grids.

The sections were incubated with polyclonal antibodies raised in rabbits to guinea pig BSP. Positive immunostaining was detected by protein A coated with 10 nm gold.

The distribution of immunogold markers for BSP was studied by systemic random sampling on printed copies at a final magnification of 65,000. Three compartments were defined in the proximal epiphysis, represented by mineralized cartilage, bone/cartilage interface and subchondral bone. For comparison, background levels of immunolabeling was measured in the osteoblast nuclei. The osteoarthrotic medial plateau was compared with the light microscopically non-OA lateral condyle in the same knee. Control sections were incubated with rabbit serum.

Results (Table 1)

The central non-meniscus covered portion of the medial plateaus showed evidences of OA with a rough cartilage surface and osteophytes at the joint margins. The surface on the lateral side was smooth without any signs of OA. BSP labeling was highest at the osteocartilaginous interface in both medial and lateral condyles. Labeling decreased in bone with increasing distance from the interface. The marker distribution was at background levels in cartilage in both groups. There were no differences between OA and control groups. Distribution in control sections was negative.

Discussion

In previous morphological studies, we have described qualitative and quantitative histological changes in the guinea pig OA model with high incidence of medial gonarthrosis, histologically close to human OA, occurring between 6 and 12 months of age. Subchondral bone changes seems to occur simultaneously with cartilage changes. Our results have given further support to previous ideas that subchondral bone changes may be important in the pathogenesis of OA (Radin 1986).

Table 1. Mean values *SD* for goldmarkers per area unit (n=3)

The osteocartilaginous border has a coral-like appearance, with a surface about 5–6 times the articular surface. Its irregular and serrated border is obviously the result of an ongoing remodeling. The surface density (Sv) of the osteocartilaginous interface decreases in the medial plateau with increasing OA (de Bri, unpublished), which may be a result of cartilage extensions into the subchondral bone partly being resorbed along with the remodeling.

We observed a BSP accumulation in the osteocartilaginous interface in both the medial plateau with advanced OA, and the lateral plateau with no histological evidences of OA. There was no difference in BSP labeling between the condyles. These results confirm previous findings from our lab of an increased BSP distribution in the osteocartilaginous interface (Hultenby 1994). It is likely that BSP binds to other components at the very interface, thus becoming fixed at this site. Also, it is likely that the molecule has a structural and/or regulating role at the interface, possibly as an anchor of calcified articular cartilage to subchondral bone or by regulating mineralization at the ostecartilaginous interface.

Further studies of the BSP labeling in different stages of OA may reveal alterations in the BSP labeling during destructive and proliferative OA phases.

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