Histopathological Findings on Ulcerative Lesions of Carpal and Tarsal Joints in Japanese Black Cattle

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ABSTRACT. In order to determine the pathogenesis of ulcerative lesions of the articular cartilages in Japanese Black cattle, tissue samples of the ulcerative lesion, marginal portion of the ulcer, macroscopically normal portions and synovial membranes were histopathologically examined by light microscopy, scanning electron microscopy and contact microradiography. The results are summarized as follows: (1) In the ulcerative lesions, degeneration and complete destruction of articular cartilage and its replacement with a proliferation of myelogenic connective tissue were observed. (2) In macroscopically normal portions, fissures of the articular surface and changes of the trabecular pattern in subchondral bone were present. (3) In the marginal portions of the ulcerative lesions, evidences of the repair process such as connective tissue growth from subchondral bones and articular cartilages were seen. (4) In synovial membranes, no pathological findings were observed. And (5) among the above mentioned changes, no inflammatory findings were seen. In conclusion, the ulcerative lesions of bovine articular cartilage may be regarded as the early stage of osteochondrosis to osteoarthrosis since the findings such as non-inflammatory destruction or degeneration and remodeling of the joints are characteristics of the latter. — KEY WORDS: carpal and tarsal joints, cattle (Japanese Black), histopathological study, ulcerative lesion.

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It has been previously reported [18] that ulcerative and/or erosive lesions of articular cartilages at many joints were observed in large number of Japanese Black cattle. Moreover, the highest incidence was present at the medial half of the carpo-metacarpal joint, the caudo-lateral half of the tarso-metatarsal joint and the interphalangeal joint [18]. Although similar lesions were reported by Bauer *et al.* [1], and Benett and Bauer [2], its clinical importance remains unknown because the affected cow rarely has clear clinical signs such as lameness, pain or swelling of the joints. There are many reports [3, 16, 19, 20] on bovine joint diseases and various terms such as osteoarthritis, osteoarthrosis (OA), degenerative joint disease or osteochondrosis (OC) [22] have been used, however the lesions may be similar to those seen in Japanese Black cattle [18]. But the relationship of OC to OA in bulls is unknown [22].

In this study, histopathological examinations were conducted on the lesions of the carpal and the tarsal joints of Japanese Black cattle in order to clarify the pathogenesis of this lesion.

MATERIALS AND METHODS

Animals: Fifteen carpo-metacarpal and three tarso-metatarsal joints were obtained from eight slaughtered Japanese Black steers at 3–5 years of age and 590–685 kg of body weight. Tissue specimens were obtained from the articular cartilage with subchondral bone of the ulcerative portion, marginal portion of the ulcer and macroscopically normal portion, and synovial membranes in order to do histological examinations by light microscopy, scanning electron microscopy and contact microradiography.

Light microscopy (LM): All specimens were fixed in 10% buffered formalin. These tissue blocks were decalcified using the Plank-Rycho method and embedded in paraffin. Four micrometer sections were made and stained with hematoxylin-eosin (HE), Masson trichrome, 0.1% toluidine blue (pH 4.1) and Periodic-Acid-Schiff (PAS) reaction.

Contact microradiography (CMR): Undecalcified specimens were prepared from polyester blocks according to the modified technique for grinding specimens, stained with tetrachrome, and radiographs were taken by a soft X-ray apparatus (SOFRON®, SROM40S, Soken, Tokyo) using Kodak film (No. 649–0). The optimun exposure was found to be 12 to 15 kVp at 5 mA and 8 to 10 min for the specimens of 40 to 50 μm thickness.

Scanning electron microscopy (SEM): All specimens were washed with normal saline solution after which thin blocks of cartilage and synovial membrane were taken. These blocks were fixed overnight in 2.5% glutaraldehyde solution and then fixed overnight in 1% osmium tetroxide solution in 0.1 M cacodylate buffer at pH 7.4. They were dried at a critical point, coated with gold and examined using an Alpha-10 scanning electron microscope (Akashi, Tokyo).

RESULTS

The characteristic histopathological findings of the affected joint with regards to its ulcerative portion, macroscopically normal portion, marginal portion of the ulcer and synovial membranes are described below.

Ulcerative portions: The most characteristic findings in CMR of the ulcerative parts were thinning of the trabeculae of subchondral bone, with increased inter-trabecular space

and change in pattern of trabecular orientation due to the depression of articular surface and loss of the normal trabecular bone orientation with persistence of horizontal structures (Fig. 1-a). On the other hand, in the more severely eroded deep ulcers, the underlying trabeculae was thinner than that of the shallow ulcer. Moreover, the vertical structure was drastically reduced in size. In less distinctive ulcers, osteosclerotic findings with adhesion of bone trabeculae were observed. These findings were observed in all the specimens.

Under LM, the ulcerative lesions involved apparent denudation and defect of articular cartilage with exposure of subchondral bone, and its replacement with a myelogenic vascular bud (Fig. 1-b). Furthermore, osteosclerosis of subchondral bones and numerous fat cells within the fibrous tissue and the formation of subchondral bone cysts (Fig. 2-a) were present. However, there was no infiltration of inflammatory cells.

By SEM, large or small crater-like concaves, hive-like small foramina and many fissures with comparatively smooth surfaces were observed on the surface of the ulcer. No articular cartilage was found in the ulcerative portion which was destroyed and replaced by a network-structure with many collagen fibers and fibrous cells of myelogenic tissue (Fig. 2-b).

Macroscopically intact portion: Under LM, articular cartilages of the macroscopically intact portion was composed of hyaline cartilage with a smooth surface. On the surface the paired cartilage cells, which were two to four layers deep beneath the surface layer, the cells were grouped within lacunar spaces in clusters. The cartilage matrix appeared homogeneous with no evidence of fibrillation. However, the deeper subchondral bones were slightly osteosclerotic with thickened trabeculae. Osteons were variable in size with narrow Haversian canals and cementum lines were irregular. According to the results of the CMR and tetrachrome staining, it was recognized that subchondral bone trabeculae were reduced in size.

Other abnormal findings in macroscopically intact portions included a wedge-shaped depressive chondral lesion in two animals (Fig. 3-a) and pedunculated proliferation of subchondral tissues. The former lesion was more extensive on the articular surface than the latter. The remaining cartilage appeared to be fibrocartilage and was invaded by blood vessels from the inter-trabecular spaces of subchondral bone. The surface cells formed clusters and also the surrounding matrix formed an accentuation of metachromasia. In the latter, ossification and depression of the adjacent bone tissue of pedunculated proliferation were observed and the entire trabeculae were rearranged by absorption and intention.

By SEM, cartilage with numerous intertwined collagen fibers and the stripping of the superficial layer was observed in a few specimens of macroscopically intact portions (Fig. 3-b). In other cases, the superficial tissue had completely disappeared and a hive-like structure with empty lacunae due to the absence of superficial cartilage cells was seen.

Marginal portion: Under LM, both regression or complete disappearance of the cartilage with less metachromasia and the destruction of subchondral trabeculae were observed in the marginal portion of ulcerative lesions. Most of the cartilage appeared to be fibrocartilages which was derived from the intertrabecular spaces of the subchondral bones beyond the tidemark. The cartilage cells were immature and were in clusters, similar to the deeper layer of normal cartilage. Vertical collagen fibers were also observed (Fig.

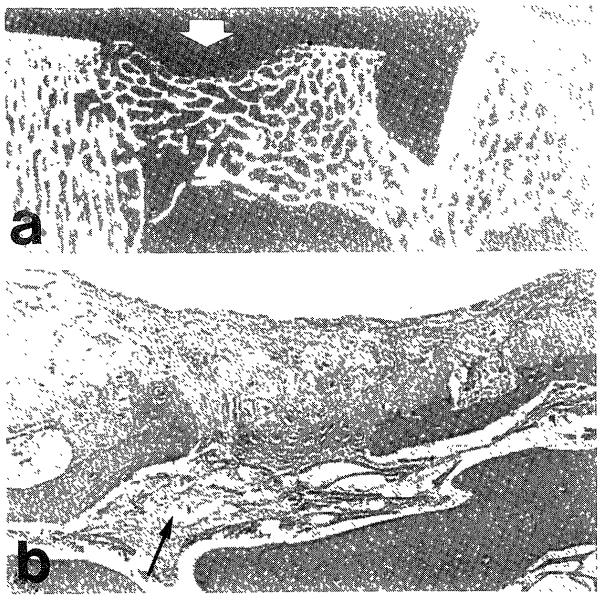

Fig. 1-a. CMR of the ulcerative lesions (arrow) shows a severe destruction, deficit, or rearrangement of subchondral bone trabeculae, and depression of the articular surface. Undecalcified specimen grounded, 40 μm in thickness, × 4.5.

Fig. 1-b. The ulcers are partially repaired by connective tissue with blood vessels (arrow). HE, × 100.

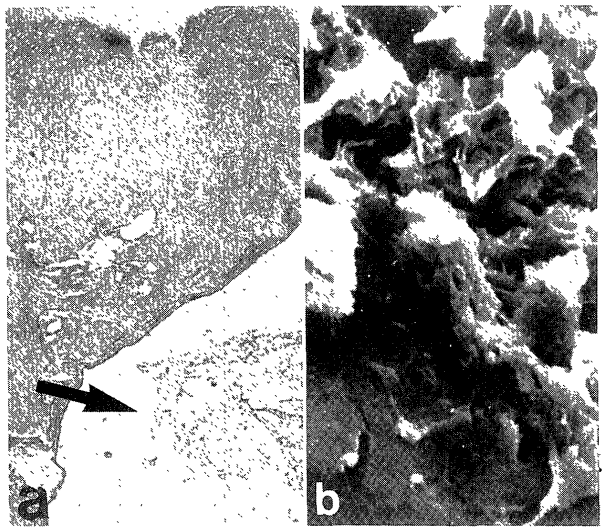

Fig. 2-a. The formation of subchondral bone cyst (arrow) is found at ulcerative portion. HE, × 100.

Fig. 2-b. Superficial tissue is destroyed, and a network-structure of collagen and fibrous cells at ulcerative portion are present. SEM, × 1,526.

4).

Under CMR, less subchondral bone was present and the underlying bone trabeculae were thinned with realignment to orientation of the ulcer.

By SEM, network structure of collagen tissue and fibrous cells from the normal portion to the ulcerative lesion were observed (Fig. 5). These fibrous tissues crossed each other and were located on large or small foramina and penetrated the normal articular surface.

Synovial membranes: Under LM and SEM, no significant pathological lesions were found in the synovial membranes. Under LM, two to three layers of paired lining cells on the surface and loose connective tissue with numerous blood vessels beneath the surface layer were present.

DISCUSSION

From an etiological point of view, bovine joint diseases with various lesions of the articular cartilages are generally classified into two major categories; inflammatory and degenerative [1, 2, 14–18, 20]. Although the former is associated with septic or traumatic arthritis and has been well studied in cows, the significance, pathogenesis or etiology of bovine joint disease has not yet been reported. However, obesity, nutrition, genetic and metabolic factors are recognized as the important factors for the development of bovine joint disease [1, 2, 16, 20, 22].

A confirmatory characteristic finding is the articular ulcer which may occur as a result of inflammation or degeneration. Inflammation is characterized by heat, swelling, redness, pain and dysfunction in the clinical state and involves degeneration, hyperemia, exudation, cell infiltration and proliferation of tissue under histopathological examination. In this study, there were no clinical inflammatory signs nor infiltration of inflammatory cells seen under LM and SEM. Therefore, the degeneration or destruction of articular cartilages and eburnation of exposed bone observed in the ulcerative lesions suggests that these are non-inflammatory alterations. Further investigations are necessary on pathogenesis related to mechanical stress and the alterative pathogenesis to collagen damage vs proteoglycan damage as initial step.

In this study, metachromasia in the superficial matrix decreased in most of the lesions as seen by toluidine blue staining. This finding suggests the increased alteration in glycosaminoglycans (GAG) content because metachromasia is associated with the presence of GAG. However, the cartilage matrix of the marginal portion in which nearly normal architecture was seen was often more intensely stained with toluidine blue. Furthermore, hypertrophy and glomeration of chondrocytes were also seen along the fissure

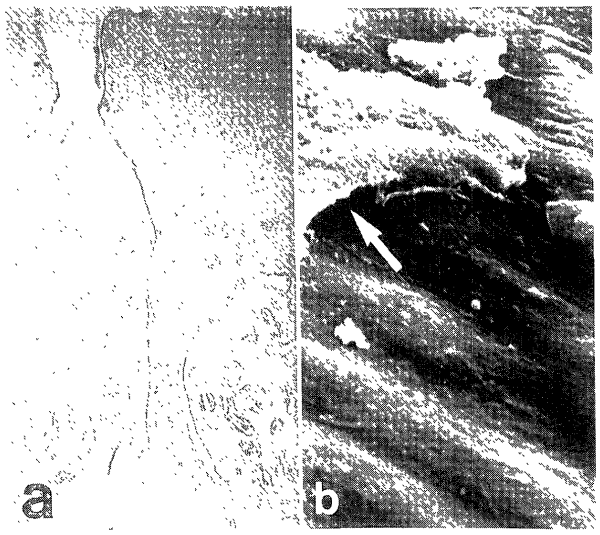

Fig. 3-a. A wedge-shaped depressive chondral lesion is found in macroscopically intact portion by light microscopy. The remaining cartilage appears as fibrocartilage and it is invaded by blood vessels from the inter-trabecular spaces of subchondral bone. HE, × 100.

Fig. 3-b. The stripping (arrow) of the superficial layer has progressed in to macroscopically intact portion. SEM, × 540.

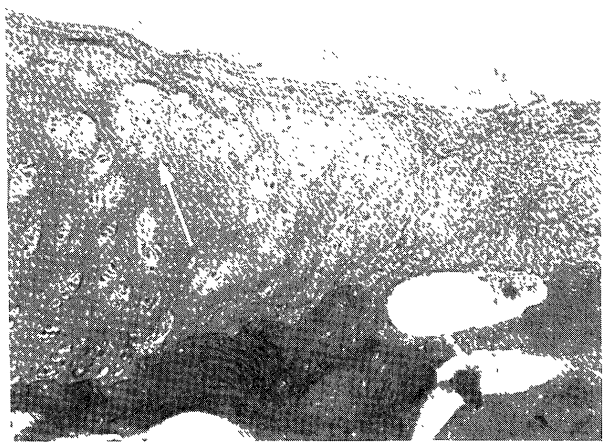

Fig. 4. Multiple clones of cartilage cells (arrow) are present in the transitional zone at the margin of ulcer. HE, × 100.

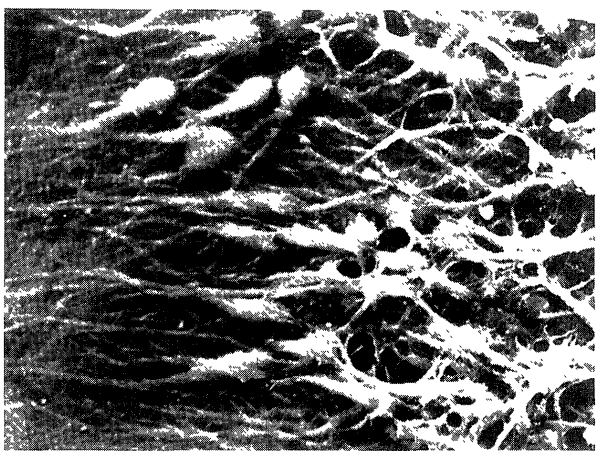

Fig. 5. Transitional figures are observed from intact portions with relatively smooth structure to the ulcerative lesion with a network structure of collagen tissue and fibrous cells by SEM. × 728.

of the cartilage as a response to the lesion. Striking changes of bone trabeculae and osteosclerotic findings or eburnation of various bone cysts were observed in deeper area of the subchondral bone.

The lesions observed in this study are quite similar to the degenerative changes reported by Bauer *et al.* [1], or Benett and Bauer [2] and to osteochondrosis (OC) and to osteoarthrosis (OA) in human. Also, the clear synovial fluid of normal volume with normal cellular components in almost all the joint examined [19] suggests that these lesions are degenerative. These ulcerative lesions of bovine articular cartilage may be regarded as the early stage of OC to OA since the findings such as non-inflammatory destruction or degeneration of cartilage and remodeling of the joints are characteristics of the latter, though there are a few ones of the former as chondral hypertrophy and necrosis.

In the early stages of OA, there is a decrease in the amount of GAG in the superficial cartilage matrix as well as alteration of the nuclei and the disappearance of cells from lacunae [2, 13]. In more advanced cases, the chondral repair mechanism with hypertrophy and glomeration of chondrocytes may take place. Mankin and Lippiello [10] suggested that the articular cartilage of this disease was destroyed from the surface inward to the deeper portion [11]. In most of the specimens, connective tissue with the blood vessels of subchondral bone space had invaded the destroyed articular cartilages and the inapparent layer of cartilage was covered with granulative tissue. Furthermore, this lesion was observed in the central articular area, however pannus formation from synovial membranes which are usually seen in human OA was not observed. The disruption of the tidemark came from the subchondral side. Therefore, the deterioration of the articular cartilage is thought to be accelerated by both superficial cartilage and subchondral bone.

In an earlier study [18], it was suggested that the joints with predilection for these lesions might be related to weight bearing. Characteristic findings such as vertical destruction and rearrangement of subchondral bone trabeculae or bone necrosis in ulcerative lesions were severe than those of human [3, 5, 10] and horse [8, 15] strongly suggest that great weight bearing is one of the main etiologic factors in this lesion. However, genetic factors, nutrition and metabolic factors are also thought to be important in the development of OA in humans [3, 6]. Japanese Black cattle are usually fed high energy rations in narrow pens and have large bodies for their age. When physical forces are concentrated on a point of articulation, the subchondral bone compensates and is able to support the weight by a rearrangement of trabeculae [4–6, 9]. If the articular cartilageis not well supported by bony structures, degeneration of cartilage may occur. Once the lesions are initiated, weight bearing on the surrounding intact articular surface may cause the progression of the lesion.

Although the incidence of the above mentioned lesions in the breed was considerably higher in a slaughter house, the clinical incidence has not been determined yet. Further investigations are necessary on this as well as kinetic examinations.

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