Morphological Changes in Rabbit Articular Cartilages Experimentally Induced by Joint Contracture— in Association with Aging

By

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—Received for Publication, July 20, 1981—

Key Words: Light microscopy, Electron microscopy, Rabbit, Articular cartilage, Joint contracture

Summary: Morphological changes in rabbit articular cartilage induced by joint contracture were studied by light and transmission electron microscopy. The knee joint was plaster-immobilized for 3-9 weeks in the flexion position. Three different age groups of rabbits were examined, i. e. at the age of 1 month (young), 1 year (adult), and over 2.5 years (old).

In the non-treated groups, the surface of the articular cartilage of the old rabbit was undulated and less smooth than that of the young rabbit. In the old rabbit, cell density of the articular cartilage reduced, and the filamentous-amorphous surface layer increased in thickness.

In the plaster-immobilized groups, no significant change was noticed in the young rabbit. In the adult rabbit, on the other hand, remarkable changes in the articular surface, and the intercellular matrix occurred after 5-week-immobilization. In addition to those changes, chondrocytes were also affected in the 5-week-immobilized old rabbit. The damaged articular cartilage of the adult rabbit which had been immobilized for 3 weeks recovered, while the old rabbit did not.

A long-term plaster cast immobilization has routinely been employed in orthopedical therapeutics, and has caused the joint contracture with varying degrees in association with the age of patients. Several authors have reported that the immobilization of the joint induces morphological changes in the articular

cartilage of rat, mouse and rabbit, e. g. degeneration of some chondrocytes, morphological disturbance of the matrix and reduction of the cell density³⁾. However, these experiments have been carried out by light microscopy, and provide no further information to determine morphological changes in the immobilized

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joint of various ages in detail. In the present study we report the ultrastructural as well as light microscopical changes in the articular cartilage of the plaster-immobilized knee joint of the young, adult and old rabbits.

Materials and Methods

Normal rabbits, at the age of 1 month (young), 1 year (adult), and over 2.5 years (old), were used as materials. Each age group consisted of 15 individuals. The right hind leg of each animal was plaster-immobilized in the maximal flexion of the knee joint for 3, 5 or 9 weeks. As controls, the age-matched rabbits were reared for 5 to 9 weeks.

After the animals had been anesthetized with Ketalar (ketamine hydrochloride), the articular cartilage of the distal end of the femur was taken out carefully with the scalpel. A fraction of each specimen was prefixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, at 4°C for 3 hours, washed three times with the same cold buffer, postfixed with 1% OsO_4 in the same buffer, dehydrated in graded alcohols and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate, and observed with a JEM-100B electron microscope. For light microscopy, the other fraction of each specimen was embedded in paraffin. Sections at 4 to 5 μm in thickness were stained with haematoxylin-eosin.

Results

1. Morphology of the articular cartilage of the non-treated groups:

Under the light microscope, the articular cartilage of the non-treated, normal young rabbit was found to contain a smooth surface and two or three layers of flat chondrocytes in the superficial zone which

were arranged in parallel with the surface (Fig. 1). By contrast, the surface of the cartilage of the old rabbit was undulated and less smooth than that of the young rabbit (Fig. 2). In the middle and deep zones of the cartilage, round cells were distributed irregularly in the old rabbit (Fig. 2), and perpendicularly to the surface of the cartilage in the other two age groups (Figs. 1 and 3). The perpendicularly arranged cells of the young and adult rabbits were found to form many columns of several cells as has been well-known (Figs. 1 and 3). Cell density of these three zones of the old rabbit (Fig. 2) was likely to be reduced as compared with that of the young one (Fig. 1). Under the electron microscope, the flat chondrocytes in the superficial zone of the young rabbit were scattered in the matrix, and appeared to have indicated a morphological feature similar to the fibroblast. In the matrix many intercellular fibers and fibrils were arranged in parallel with the surface of the cartilage (Fig. 4). These fibers and fibrils were about 20 and 5 nm in diameter, respectively. The spherical cells were distributed in the middle zone. Each cell had a round nucleus, well-developed rough endoplasmic reticulum (r-ER) and the Golgi complex. Intercellular fibrils, approximately 5 nm in diameter, also surrounded these round cells densely (Fig. 5).

In the adult superficial zone, there were more elongated chondrocytes containing the conspicuously dilated r-ER than in the young rabbit. The karyoplasmic ratio of the cells in the adult rabbit superficial zone (Fig. 6) appeared to be larger when compared with that of the cells in the middle and deep zones (Fig. 7). The fibers and fibrils in the adult superficial matrix showed similar size and distribution to those in the young, but the filamentous, amorphous materials in the

surface layer of the adult (Fig. 8) were found to be thicker (about 0.1 to 0.2 μm) than that of the young (Fig. 4). Cells and matrix, similar to those of the young, were also observed in the three zones of the adult (Figs. 6 and 7).

In the old rabbit, morphological changes associated with aging occurred to some degree in the three zones. The filamentous and amorphous surface layer was the thickest among the three age groups. Its thickness was estimated at about 1 μm (Fig. 9). Fat droplets were frequently observed in the chondrocytes in the middle zone (Fig. 10). Intercellular fibrils, about 10 nm in diameter, surrounded the indented nucleus within the cells (Fig. 10). Intercellular matrix in all zones was likely to show similar features to that of the adult (Figs. 6-8).

2. Morphology of the articular cartilage of the plaster-immobilized groups:

All the articular cartilage in the young rabbit plaster-immobilized for 4 to 9 weeks was morphologically quite similar to that in the non-treated young rabbit under the light and electron microscopes as follows. The cartilage surface still remained quite smooth (Fig. 11) in contrast with that of the plaster-immobilized old rabbit (Fig. 12). As shown in Fig. 11, there were two or three layers of flat cells in the superficial zone and round cells in the middle and deep zones. The cell density was also similar to that of the non-treated rabbit (cf. Fig. 1). The intercellular matrix and the subcellular organelles (Fig. 13) appeared the same as seen in Figs. 4 and 5.

In the adult and old rabbits, on the other hand, striking changes occurred. After 5-week-plaster immobilization, the articular cartilage surface was not smooth any longer but remarkably undulated in profile under the light microscope (Fig. 12). The matrix in all zones of the

cartilage appeared coarser than that of the non-treated animals (Figs. 2 and 3). By electron microscopy, the felt-like amorphous material was observed in the most superficial zone of the cartilage. It constituted a thickened layer at the cartilage surface and sometimes protruded into the articular cavity (Figs. 14 and 15). Even in the superficial zone, intercellular fibers and fibrils were not regularly but randomly distributed in the matrix (Fig. 16). The structural changes as described above were slightly observed after 3-week-plaster immobilization and disappeared in the adult rabbit which was remobilized for 5 weeks after the plaster had been discarded. When immobilized for 5 weeks, the typical morphological changes were induced in each treated animal. The old rabbit, in particular, was more seriously affected as compared to the adult. In the adult rabbit, for example, some of the areas of the superficial zone of the cartilage matrix sometimes remained intact. Chondrocytes were also affected in the old rabbit plaster-immobilized for 5-9 weeks. Cells in the middle zone were not regularly but randomly distributed (Fig. 12). In the old rabbit, degenerated cells were found in the superficial zone as well as in other zones (Fig. 17). Indented nuclei, fat droplets and fibrils were observed within the cells. In the immobilized adult, the remarkable changes in the cellular elements were not encountered.

Discussion

Changes in arrangement and density of collagenous fiber bundles in the matrix of the superficial zone of the articular cartilage are induced by the immobilization of the joint and also occur in the course of aging of the rabbit^{1,2,5}. In the present study, similar changes of the plaster-immobilized leg are also observed under

the light microscope. These changes do not occur in the young rabbit, but are more intensive in the old rabbit. Under the electron microscope, these fibers are composed of collagenous fibers of about 20 nm in diameter and other fibrils of about 5 nm in diameter, and are arranged irregularly as compared with those of the non-treated rabbits. Further, the immobilization of the joint induces the amorphous substances of the surface in the cartilage to become thicker and more irregular than those of the non-treated rabbits. The thickness and irregularity of the substances are the largest in the immobilized old rabbit. Thus, it seems to depend on the age of the rabbit to what degree morphological changes in fibers and the surface substances are brought about in the leg contracted by the plaster-immobilization.

It has been described that the chondrocytes in the middle and deep zones are degraded and/or effeted with aging or immobilization, and that the cells in the superficial zone resemble fibroblasts morphologically and functionally⁴⁾. It has remained undetermined, however, whether the cells in the superficial zone are affected by immobilization or not. The present study clearly demonstrates that the cells of the superficial zone as well as those of the middle and deep zones in the immobilized adult and old rabbits are be degraded and/or effeted. Cell density in the cartilage of the non-treated old and the immobilized adult and old rabbits is also likely to be similarly reduced to that of the cartilage at the lower end of the femur of the old rabbit, as described by Barnett et al. (1963). These events imply that the plaster-immobilization

induces the degradation or effeteness and subsequent reduction of chondrocytes in all zones of the articular cartilage.

Therefore, morphological changes in the cartilage induced by plaster-immobilization, e.g. disturbances of fibrers in the matrix, degradation of chondrocytes in all zones, thickening of surface substance and changes of organelles in the cells, are suggested to depend on the aging of the rabbit, the cell activity, the mobility of the joint, the period of immobilization, and the nutritional condition of the cells after immobilization.

Acknowledgements

The authors wish to express their gratitude to Mr. M. Fukuda, Laboratory for Electron Microscopy, Kyorin University School of Medicine, for his technical assistance.

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PLATES

Explanation of Figures

Plate I

Figs. 1-10 and Figs. 11-17 show the articular cartilage from the non-treated and plaster-immobilized rabbits, respectively.

Figs. 1-3. Light micrographs (LM). H. and E. S: cartilage surface.

Fig. 1. Young rabbit. Non-treated. The cartilage surface is smooth. Two or three layers of flattened cells are seen in the superficial zone. $\times 600$.

Fig. 2. Old rabbit. Non-treated. The surface of the articular cartilage is undulated and less smooth than that of the young rabbit (cf. Fig. 1). Cell density of the cartilage is reduced as compared to that of the latter. $\times 600$.

Fig. 3. Adult rabbit. Non-treated. The perpendicular arrangement of the cartilage cells is clearly seen in the middle and deep zones. The cartilage surface is rather smooth. $\times 800$.

Fig. 4. Electron micrograph (EM) of the superficial zone of the articular cartilage. Young rabbit. Non-treated. Flattened chondrocytes are scattered in the matrix and surrounded by intercellular fibers and fibrils, most of which are arrayed in parallel with the cartilage surface (S). $\times 6,000$.

Plate II

- Fig. 5. EM. Young rabbit. Non-treated. A fully differentiated, spherical chondrocyte situated in the middle zone of the cartilage. It has a rounded nucleus, well-developed r-ER and the Golgi. It is surrounded by intercellular fibrils, approximately 5 nm in diameter. The similar type of the cells as seen in this picture is usually found in both middle and deep zones. $\times 6,000$.
- Fig. 6. EM. Adult rabbit. Non-treated. A flattened chondrocyte found in the superficial zone. An elongated nucleus and r-ER with dilated cisternae are conspicuous. The so-called karyoplasmic ratio seems larger than that of the cells in the middle and deep zones. $\times 10,000$.

Plate III

- Fig. 7. EM. Adult rabbit. Non-treated. A fully differentiated chondrocyte found in the middle zone. The cell with a rounded nucleus is surrounded by fine fibrils, about 15-20 nm in diameter. $\times 6,000$.
- Fig. 8. EM. The superficial zone of the cartilage of an adult rabbit. Non-treated. Collagen fibrils are arrayed in parallel with the cartilage surface (S). The filamentous-amorphous surface layer (indicated by arrows) is about 0.1-0.2 μm thick. $\times 20,000$.
- Fig. 9. EM. The superficial zone of the articular cartilage of an old rabbit. Non-treated. The filamentous-amorphous surface layer (indicated by arrows) is about 1 μm in thickness and much thicker than that of the adult rabbit (cf. Fig. 8). Collagen fibrils are arranged in parallel each other. $\times 20,000$.

Plate IV

- Fig. 10. EM. A chondrocyte found in the middle zone of an old rabbit. Non-treated. A fat droplet (L), intercellular fibrils surrounding the indented nucleus, the Golgi, lysosomes, and distended r-ER are seen within the cytoplasm. $\times 14,000$.
- Fig. 11. LM. H. and E. 9-week-plaster immobilized, young rabbit. The articular cartilage is nearly the same as seen in Fig. 1, in terms of the cellular composition and arrangement. $\times 600$. S: cartilage surface.
- Fig. 12. LM. H. and E. 5-week-plaster immobilized, old rabbit. The articular surface (S) is remarkably undulated. The matrix become coarser in appearance (e.g. arrows). The cell density in the cartilage is the least of the examined rabbits plaster-immobilized. $\times 530$.
- Fig. 13. EM. The superficial zone of the articular cartilage. 5-week-plaster immobilized, young rabbit. No morphological change induced by contracture is found in this picture. Compare with Fig. 4. $\times 4,000$.

Plate V

Figs. 14-16. EMs. The most superficial region of the articular cartilage of 5-week-plaster immobilized adult rabbit.

Fig. 14. The felt-like amorphous surface layer (indicated by opposing arrows) is expanded in thickness. $\times 20,000$.

Fig. 15. An amorphous protrusion projecting into the articular cavity (arrow) is found on the surface layer. $\times 10,000$.

Fig. 16. Collagen and other fine fibrils are not regularly but randomly distributed in the matrix. $\times 30,000$.

Fig. 17. EM. The superficial zone of the articular cartilage. 9-week-plaster immobilized, old rabbit. A couple of degenerated chondrocytes are seen near the cartilage surface (S). An indented nucleus and lipid droplets are conspicuous. $\times 9,000$.