Thermoanalytical and histological study of intra-articular papain-induced degradation and repair of rabbit cartilage

II. Mature animals

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Farkas, T., Bihari-Varga, M., and Biró, T. (1976). *Annals of the Rheumatic Diseases*, 35, 23–26. Thermoanalytical and histological study of intra-articular papain-induced degradation and repair of rabbit cartilage. II. Mature animals. The effect of papain treatment on the cartilage tissue of the knee joint of mature rabbits was studied by histological and thermoanalytical methods. Changes taking place in the composition of cartilage in the course of ageing were shown. As a result of papain treatment a significant irreversible destruction of the ground substance took place. Papain-induced loss of proteoglycans and the intensity of repair reactions and age-related differences are discussed.

In a previous experimental study (Farkas, Bihari-Varga, and Biró, 1974) repeated intra-articular papain injections were applied to immature rabbit knee joints, similar to that described by Bentley (1971), to attempt to introduce progressive degenerative changes resembling osteoarthritis. It was then decided to repeat the experiment using mature animals. In a previous study the effect of normal ageing on the composition of rabbit articular cartilage was shown.

Material and methods

AGE-RELATED CHANGES

Hyaline cartilage was excised from the knee joints of rabbits aged 3, 6, 12, 24, 36, and 48 months. Each age group consisted of 10 animals. Fresh tissue samples were cut into small pieces and lipids removed by extraction at room temperature with 25% ether in ethanol for 2 days followed by 50% chloroform in methanol for 2 days, and were then dried. Chemical determinations were performed using a complex thermoanalytical method (Paulik, Paulik, and Erdey, 1958). The samples of approximately 50 mg were weighed into a platinum crucible of a Paulik-Paulik-Erdey MOM derivatograph. Heating rate was 2°/min up to 900°C. By means of thermal analysis, the amount of water content bound chemically to the ground

substance, the proteoglycans, and the concentration of sulphated acid glycosaminoglycans (GAGs) and of keratan sulphate were measured (Bihari-Varga, 1971a) and the structural stability of the proteins demonstrated (Bihari-Varga, 1971b) simultaneously in the same tissue sample.

ALTERATIONS CAUSED BY PAPAIN TREATMENT

Twenty-five 3-year-old rabbits of both sexes weighing 2.5 g were used. They were housed under normal conditions and fed a stock diet. None was pregnant during the experiment. 0.4 ml of a 3% papain solution (dissolved in physiological saline containing 0.75 mol/l cysteine) was injected into the knee of the right hind limb of the animals three times at 48-hour intervals. The same volume of sterile isotonic saline was injected into the opposite knee joint to serve as a control. Five animals were sacrificed at days 2, 7, 13, 35, and 42 after the third injection. A 2 mm-thick osteochondral layer was removed from the largest extension of the femoral condyle for histology. Decalcified paraffin sections were prepared and stained with haematoxylin and eosin (H. and E.), Periodic Acid-Schiff (P.A.S.), and Alcian blue.

Histological and histochemical observations were evaluated using a system, based on that of Mankin (1973), of scoring points for alterations in the structure, cellularity, nuclear and matrix staining, and the 'tide-mark' integrity. Histological features interpreted as signs of regeneration, such as mitotic figures, cloning, and focal increase of

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metachromasia around chondrocytes, scored negative points. The final score gave in every case an index of severity in cartilage alterations.

On the paraffin sections the middle one-third only of the cartilage layer was assessed, which corresponded to the contact area of the joint. The two lateral parts adjacent to the marginal and foveal synovial membranes were omitted, these being sites of degenerative changes unrelated to the experiments.

Results

AGE-RELATED CHANGES

As shown in Fig. 1 the structural water content of the ground substance and the concentration of chondroitin sulphate decreased significantly with age in rabbit hyaline cartilage. The amount of keratan sulphate showed a slight but not significant tendency to increase.

ALTERATIONS CAUSED BY PAPAIN TREATMENT

Morphological findings

On sections obtained from animals sacrificed 2 and 7 days after the last papain injection degenerative changes were found to be similar to those occurring in young animals (Farkas and others, 1974). Surface fibrillation with loss of nuclear and matrix staining and occasional deep erosions indicate severe damage to the cartilage at this period. On the sections obtained 2 weeks after papain treatment, in contrast to the

FIG. 1 Age-related changes in the ground substance of rabbit articular cartilage. ○ = water content bound structurally to proteoglycans; ● = CSA concentration (mean $\pm$ SD)

series with immature animals, the signs of regeneration did not persist but further deterioration occurred with gradual progression up to the 6th week. Photomicrographs prepared 6 weeks after the last papain injection show excessive fibrillation (Fig. 2) and erosion (Fig. 3) of the articular cartilage with loss of nuclear and matrix stain uptake. On the section illustrating the marginal areas of a deep erosion pannus is shown creeping over the surface of damaged cartilage. Scattered foci of regeneration with cluster formation are present. A similar pattern

FIG. 2 Articular cartilage from knee joint of mature rabbit 6 weeks after papain treatment. Extreme fibrillation. Haematoxylin and eosin. $\times 17$

FIG. 3 Articular cartilage from knee joint of mature rabbit 6 weeks after papain treatment. Marginal area of a deep erosion. Haematoxylin and eosin. $\times 17$

of focal regeneration is shown in the cartilage stained with P.A.S.-Alcian blue (Fig. 4). In the severely damaged cartilage metachromasia appeared only around the scanty surviving and multiplying chondrocytes.

Thermal analysis

As shown in Fig. 5 structural water content increased by about 20% in the first 2 days after papain injection, decreasing gradually after 48 hours and reaching the lowest value at the end of the first post-treatment

week when structural hydration slowly became normal. On the other hand, papain treatment initiated dramatic alterations in the proteoglycan components of the ground substance. Within the first 48 hours depolymerization of the protein moiety of the proteoglycan molecules was indicated by a significant decrease of their thermal stability. The split GAGs were then eliminated from the tissue, resulting in a 54% loss of GAG concentration by the end of the first week. During the second week, probably as a result of the compensating effect of increased GAG

FIG. 4 Articular cartilage from knee joint of mature rabbit 6 weeks after papain treatment. Scanty, focal regeneration. Periodic acid-Schiff and alcian blue. $\times 17$

FIG. 5 Papain-induced changes. A In the severity of morphological alterations, B in the structural water content of the ground substance, and C in the GAG concentration of mature articular cartilage (mean $\pm$ SD)

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synthesis, a gradual increase could be shown in the GAG level. Nevertheless, from this time until the end of the 6th week a stagnation took place in the reparative process and the GAG pattern in the injured cartilage did not reach that of the healthy tissue.

It is of interest that an excellent negative correlation could be established by regression analysis between the index of severity of histological alterations and the amount of GAGs present in the tissues ($P < 0.01$; Fig. 5).

Discussion

Papain treatment resulted in severe deterioration of the hyaline cartilage of aged rabbits as shown by morphological investigation. In a previous study performed on immature animals degenerative alterations of similar extent could not be observed. Furthermore, signs of reparative processes were found to be focal and scanty, when comparing the histological appearance with that of young animals.

Results of thermal analysis confirmed the morphological findings: papain digestion caused severe depolymerization of the ground substance and loss of proteoglycans. The compensating effect of repair reactions proved to be insufficient to recover the papain-induced degradation: the GAG after 6 weeks concentration was 33% lower than in the untreated control tissue. The decrease of GAG concentration in the course of normal ageing and the decrease in cell count after maturation (Mankin, 1968) might contribute to the discrepancies between the reaction of immature and mature animals to papain exposure *in vivo*.

Our results with regard to age-related tissue alterations seem to support the generally accepted practice of using mature animals in attempting to produce experimental arthritis (Gardner, 1960).