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PAPAIN-INDUCED MITOSIS OF CHONDROCYTES IN ADULT JOINT CARTILAGE

An Experimental Study in Full-Grown Rabbits

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When diluted papain is injected intra-articularly into the knee joints of adult rabbits, slowly progressing, degenerative changes develop in the articular cartilage. After intravenous injection of crude papain no significant degenerative changes are found. With the aid of radioactive thymidine, it is possible to demonstrate that the chondrocytes recover their ability to divide before any degenerative changes can be found. At the same time the matrix loses its staining properties with Safranin-O and toluidine blue.

Key words: cartilage; chalones; mitosis; osteoarthritis; papain

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Proteolytic enzymes break down the matrix of articular cartilage and release chondroitin sulphate (Bryant et al. 1958). Papain is a proteolytic enzyme which in articular cartilage degrades the protein polysaccharide complexes of the ground substance and diminishes the amount of glycosaminoglycans (GAG) without affecting collagen proteins (Farkas et al. 1974). The release of chondroitin sulphate in articular cartilage begins almost immediately after the administration of papain and can be studied in the cartilage as well as in blood and urine (Bryant et al. 1958). One week later, the synthesis of GAG begins again, studied by SO^4 incorporation, which is increased not due to an increased number of synthesizing chondrocytes but to an increased rate of synthesis (McElligott & Potter 1960). The GAG content of young animals, (rabbits) is restored to normal in 6 weeks, but in adult rabbits the GAG content never returns to normal (Farkas et al. 1976).

Papain administered intravenously or intra-articularly has been shown to cause degenerative and/or necrotic changes, similar to those seen in osteoarthritis, in the articular cartilage of animals (Murray 1964, Bentley 1971, Farkas et al. 1974, 1976, Havdrup & Telhag 1977).

When degenerative changes in the articular cartilage develop in man or animals, the chondrocytes recover their ability to divide (Hulth et al. 1972, Telhag 1972). It has also been shown that the chondrocytes begin to divide also when there are no histological signs of degeneration in the cartilage (Telhag 1972).

The aim of the present investigation was:

1. to study the histological and autoradiographical effect on adult articular cartilage of intra-articularly injected diluted papain (mitotic activity)
2. to study the effect of intravenously injected crude papain on adult joint cartilage.

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MATERIAL AND METHODS

The material consisted of 34 adult rabbits with roentgenographically closed epiphyseal lines. They were caged under normal conditions and fed on a normal diet. The rabbits weighed 2,085–5,100 g (mean 4,046 g). They were divided into three groups. The first (14 animals) received 0.2 ml of a 5 per cent solution of concentrated, sterile papain in the right knee joint. The left served as a control and was injected with 0.2 ml of 0.9 per cent physiological saline. The animals were killed 3 (2 animals), 7 (5 animals), 10 (2 animals) and 14 (5 animals) days after the injection. The second group (10 animals) was injected in the right knee joint with 0.2 ml of a 10 per cent solution of concentrated, sterile papain. The left knee joint served as a control and was treated as in the first group. The animals were killed 3, 6, 10, 14 and 28 days (two at a time) after the papain injection. The third group (10 animals) was injected intravenously with crude papain* in a 1 per cent solution, 10 mg/kg body weight. One week later, half of the group received another intravenous injection of papain in a 1 per cent solution, 20 mg/kg body weight. Two weeks after the first injection the animals were killed. All the animals in all three groups received 40 μCi ^{3}H-thymidine intra-articularly into both knee joints 24 hours before sacrifice.

The animals were killed with an overdose of Nembutal® intravenously (Abbot). None of the joints showed any signs of infection. Both knee joints were dissected and fixed in 10 per cent formalin solution. The patella, tibia and femur were dissected free and treated separately. The tibia and the femur were divided into two halves in the frontal plane with a circular saw. The pieces were decalcified in 50 per cent formic acid and 20 per cent sodium citrate. They were embedded in paraffin and cut into sections 5–7 μ thick.

The sections were stained with haematoxylin-eosin, Safranin-O and toluidine blue (pH 4.2). Autoradiograms of routine histological sections were prepared from both knee joints according to the dipping method with Ilford K2 liquid emulsion. The autoradiograms were exposed for 3 weeks, after which they were developed in Gevaert X-ray developer G 230 and fixed in Gevaert X-ray fixer G 350. The sections were stained through the emulsion with Mayer's haematoxylin. As a rule, 15–20 autoradiograms from each knee joint were studied, the number of autoradiograms being evenly distributed between the tibia, femur and patella.

*Papain was obtained as a crude powder derived from papaya juice from BDH Chemical Ltd.

RESULTS

Histological examination

Injected joints: 5 per cent papain

Three and 7 days after the injection proliferation of cells and osteoblastic activity were seen at the margins. In one specimen, 7 days after the injection, there was disturbance of the normal arrangement of the chondrocytes and fibrillation of the superficial and transitional layers. Reduced staining or no staining at all with Safranin-O and toluidine blue was seen.

Ten and 14 days after the injection proliferation of cells and osteoblastic activity were seen at the margins. In three specimens, one 10 days and two 14 days after the injection, flaking of the superficial layer and fibrillations down in the transitional layer were seen. Sometimes the normal arrangement of the cells was disturbed and dead chondrocytes could be seen. In the matrix, there was reduced staining or no staining at all, using Safranin-O and toluidine blue.

Control knee joints (after injection of 5 per cent papain in the right knee). None of the animals showed any signs of degeneration. In two animals (6 and 10 days after the injection) there was proliferation of the cells in and near the periosteum and perichondrium at the margins as well as osteoblastic activity. In another animal (7 days after the injection) slight subchondral osteoblastic activity could be seen. Staining of the matrix with Safranin-O and toluidine blue was normal.

Injected joints: 10 per cent papain

Three and 6 days after the injection proliferation of cells in and near the periosteum and perichondrium was seen as well as some osteoblastic activity, sometimes subchrondrally. Reduced staining or no staining at all was found, when using Safranin-O and toluidine blue.

Ten, 14 and 28 days after the injection the same changes were seen at the margins as described in sections 3 and 6 days after injection, but the changes were even more pronounced. In two animals, one 14 and one 28 days after the injec-

Table 1. Papain 5 per cent. (Left knee in brackets)

Time after injection in the right knee	No. of labelled cells	No. of histological sections	Labelled cells/ number of sections
3 days	0 (0)	43 (49)	0 (0)
7 days	2 (0)	52 (55)	0.04 (0)
10 days	28 (3)	43 (34)	0.65 (0.09)
14 days	36 (20)	48 (58)	0.75 (0.35)

tion, fibrillations down in the columnar layer, death of the chondrocytes and cluster formation were seen. There was reduced staining or no staining at all of the matrix when using Safranin-O and toluidine blue.

Control knee joints (after injection of 10 per cent papain in the right knee). No degenerative changes were seen in the articular cartilage. In one animal (3 days after the injection) slight proliferation of cells at the margin was seen. Staining with Safranin-O and toluidine blue was normal.

Crude papain, intravenously injected

In two animals, small residues of the epiphyseal line were found, but a well defined "tidemark" was seen in all animals. In one specimen (injected once), dead chondrocytes, flaking of the superficial layer, fibrillation down in the columnar layer and cluster formation were found.

In the animals injected once with papain intravenously, staining with Safranin-O and toluidine blue was normal. In the group given two injections there was no staining or reduced staining with Safranin-O and toluidine blue.

Autoradiography

Five per cent papain

A constant rise in the number of labelled chondrocytes per section was seen up to 14 days after the injection (Table 1).

Ten per cent papain

The number of labelled chondrocytes per section increased up to 14 days after the injection. Twenty-eight days after the injection, a decrease in the number of labelled chondrocytes was found (Table 2).

Control knee joints

Tritium-labelled chondrocytes were seen in four knee joints, two in each group (5 and 10 per cent papain injected). In the group injected with 5 per cent papain one animal had labelled chondrocytes in the cartilage with areas of dead chondrocytes in the columnar layer and in this cartilage also cluster formations were seen (10 days after the

Table 2. Papain 10 per cent. (Left knee in brackets)

Time after injection in the right knee	No. of labelled cells	No. of histological sections	Labelled cells/ number of sections
3 days	0 (4)	52 (47)	0 (0.09)
6 days	5 (1)	41 (41)	0.12 (0.02)
10 days	53 (0)	42 (42)	1.26 (0)
14 days	194 (0)	38 (43)	5.11 (0)
28 days	50 (0)	42 (46)	1.19 (0)

Table 3. Crude papain

	No. of labelled cells	No. of histological sections	Labelled cells/ section
One injection	122	220	0.61
Two injections	78	204	0.38
			P n.s.

injection). The other animal in this group had labelled cells in histologically normal cartilage (14 days after the injection). In the group injected with 10 per cent papain, labelled cells were found 3 and 6 days after the injection in normal cartilage (Tables 1 and 2).

Crude papain intravenously

Labelled chondrocytes were found in both knee joints in all the animals but one, and that one had received two injections of crude papain. There was no statistically significant difference between the animals that had received one and those having two injections intravenously (Table 3).

DISCUSSION

Intra-articularly administered papain results in changes in the articular cartilage of experimental animals similar to those seen in osteoarthritis (Collins 1949, Bentley 1971, Farkas et al. 1974, 1976, Havdrup & Telhag 1977). The use of concentrated papain gives rise to a very rapid degeneration and/or necrosis of the articular cartilage and as early as 2 days after injection pronounced changes are seen (Havdrup & Telhag 1977). When using a 20 per cent solution the degeneration does not develop so quickly. In both cases mitosis of the chondrocytes occurs; this can possibly be explained as an attempt to repair the articular cartilage. When using 5 and 10 per cent solutions of papain relatively small degenerative changes are seen in the cartilage, and these mostly occur focally in the form of areas with dead chondrocytes (Figure 1), flaking and fibrillations, but with parts of the cartilage remaining normal histologically. The staining properties of the matrix with Safranin-O and toluidine blue were reduced or there was no staining at all, probably due to a reduction in the amount of glycosaminoglycans or to reduced synthesis. In the control knee joints the staining properties were normal.

When crude papain is injected intravenously the concentration of papain in the joint cavity seems to be low and no significant degenerative changes are found. The concentration of crude papain in the joint seems to be sufficient to initiate mitotic activity, even when only minor or no degenerative changes at all are seen in the articular cartilage. This is in agreement with earlier findings in experimental osteoarthritis (Telhag 1972). The staining properties with Safranin-O and toluidine blue were normal after one injec-

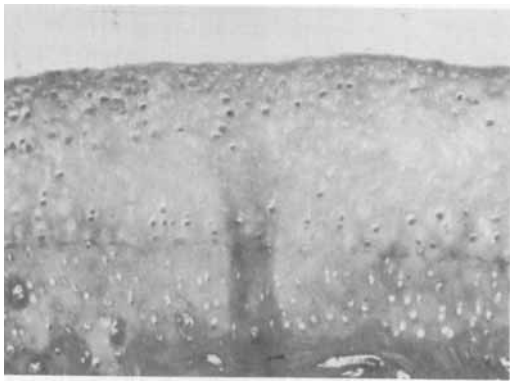

Figure 1. Two weeks after injection of 10 per cent papain. Focally, death of the chondrocytes in the articular cartilage. Objective ×6.3.

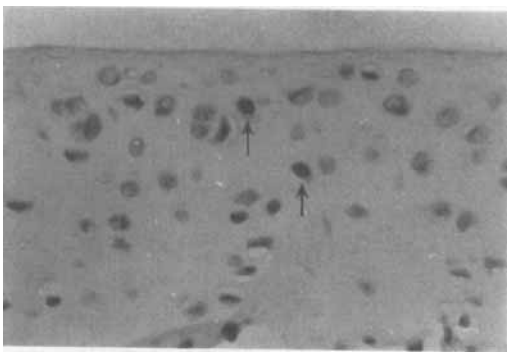

Figure 2. Two weeks after injection of 10 per cent papain. Microautoradiogram with thymidine-labelled chondrocytes without any significant signs of degeneration of the articular cartilage. Objective ×16.

tion but reduced or absent after two, which seems to be due to the fact that one injection is not sufficient to influence the matrix, but two will reduce the amount and the synthesis of glycosaminoglycans.

At the margins, proliferation of cells in and near the periosteum and perichondrium was seen, more noticeable after higher concentrations of injected papain, and increasing with time after the injection. This has been shown in earlier investigations (Bentley 1971, Havdrup & Telhag 1977).

Mitosis of chondrocytes in normal, non traumatized, adult joint cartilage has never been demonstrated in animals or human beings with certainty (Mankin 1963, 1964, 1968, Hulth et al. 1972, Telhag 1972, 1973). When concentrated papain and papain in a 20 per cent colution were used (Havdrup & Telhag 1977), mitoses of chondrocytes in the contralateral control knee joint were sometimes seen. In these joints the staining properties of the matrix with Safranin-O were sometimes absent which might possibly be due to a minute portion of papain having been transported to these joints. After giving 5 and 10 per cent papain, no reduced staining was found in the cartilage of the control knee joints, perhaps because the amount of papain being transported to these joints was too small to affect the glycosaminoglycans.

Local traumatization of cartilage results in mitoses of the chondrocytes also in the absence of degenerative changes in the articular cartilage (Havdrup et al. 1975, Havdrup & Telhag 1978). The same results are found after intra-articular injection of trypsin (Havdrup 1979). In the present investigation mitoses of chondrocytes were found without any significant signs of degeneration (Figure 2). When 5 and 10 per cent papain was used, an increasing number of labelled chondrocytes were found up to 14 days after the injection, but after 28 days, when 10 per cent papain was injected, the mitotic activity decreased.

Labelled chondrocytes were seen 3–6 days after the injection of papain, which is in agreement with the findings after operation on knee joints to produce degenerative joint disease (Telhag & Lindberg 1972).

This investigation shows that injection of papain produces mitoses of the chondrocytes in the joint cartilage both in the injected joint and, to a certain degree, also in the contralateral one. Papain damages the proteoglycan molecules resulting in release of the glycosaminoglycans from the protein core. The occurrence of mitoses might be an attempt to repair the damaged cartilage. It is also possible that papain removes mitosis-inhibiting molecules from the cell membranes. Perhaps these molecules could be the "chalones" described by Bullough & Laurence (1960), but this question cannot be answered by the above experiment.

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