BY INVITATION

EFFECT OF INTRA-ARTICULAR PAPAIN, ACETYLSALICYLIC ACID, INDOMETHACIN, PROSTAGLANDIN AND ALCOHOL ON THE ARTICULAR CARTILAGE OF RABBIT KNEES.

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SYNOPSIS

The present study showed that papain, indomethacin and alcohol when injected intra-articularly in rabbit knees produce depletion of the glycosaminoglycans of the articular cartilage with progressive cartilage damage demonstrable roentgenographically and by histochemical methods. Contrary to the observations of other authors, similar cartilage changes were not observed after intra-articular injections of acetylsalicylic acid and prostaglandin B_2.

It was concluded from this study that,

i) intra-articular injections of papain produce a good experimental model of osteoarthritis in rabbits for further studies of degenerative joint disease.
ii) although the anti-inflammatory drugs by virtue of their anti-phlogistic properties control inflammation and tissue damage in chronic inflammatory arthritis, they may perpetuate the arthropathy when used in osteoarthrosis due to their inhibitory action on the synthesis of glycosaminoglycan of the cartilage matrix.
iii) although prostaglandins are known to produce inflammatory reaction, it is still not certain whether they inhibit glycosaminoglycan synthesis.
iv) absolute alcohol if applied to the articular cartilage, for example during debridement of open wounds of the joints, may lead to serious cartilage damage and arthropathy.

Human osteoarthrosis is characterised in the early stages by degradation of the articular cartilage matrix followed by proliferation of mature articular cartilage chondrocytes from the deeper layers in response to the loss of the matrix. Lesions of articular cartilage simulating human osteoarthritis have been produced in experimental animals in the past by scarification (Meachim, 1963), by continuous compression (Salter and Field, 1960; Trias, 1961; Thompson and Bassett, 1970; Ginsberg, 1970), by creating surgical instability (Ehrlich et al, 1975) and by intra-articular injection of papain (Sweet and Solomon, 1968; Bentley, 1971, 1975). The lesions created by surgical instability and intra-articular injections of papain closely resembled the articular cartilage lesions seen in human osteoarthritic joints. In recent years it has been shown that although many anti-inflammatory drugs like salicylates, indomethacin, ibuprofen and hydrocortisone control joint inflammation and tissue damage in chronic inflammatory arthritis, they tend to inhibit synthesis of glycosaminoglycan of the cartilage matrix leading to articular

cartilage degeneration (Domenjoz, 1971; McKenzie et al, 1976). The wide-spread use of these drugs in human osteoarthrosis, a malady not characterised by an inflammatory response may therefore be harmful to the articular cartilage. Similarly, although prostaglandins E_1, E_2 A_2, B_2 and F_2 when administered intradermally have been known to suppress adjuvant arthritis in mice (Aspinall and Cammarata, 1969; Aspinall et al, 1969; Zurier and Quagliata, 1971; Zurier and Ballas, 1973), prostaglandins A_1 and B_1 have been shown to inhibit cartilage synthesis in embryonic chicken cartilage (Eisenbarth and Lebovitz, 1974; Kirkpatrick and Gardner, 1976) and intra-articular injections of prostaglandin E_1 in rabbit knees have been known to produce articular cartilage degradation and synovitis (Teitz and Chrisman, 1975).

The aim of this study is to observe by histochemical methods the changes produced in the articular cartilage of rabbit knees after intra-articular injections of papain, acetylsalicylic acid, indomethacin, prostaglandin B_2 and seventy percent alcohol.

MATERIALS AND METHODS

Intra-articular Injections

Twenty laboratory bred white rabbits of average age of four months and average weight of 1.78 kilogramme were divided into five groups. The right knee of each rabbit was injected without any anaesthesia in Group I with 0.2 ml. of 4% papain with 0.1 ml. of 0.03M Cysteine, in Group II with 0.3 ml. of indomethacin (500 ug/ml) dissolved in 5% alcohol, in Group III with 0.3 ml. of acetylsalicylic acid (5 ug/ml), in Group IV with 0.3 ml. of prostaglandin B_2 (333 ug/ml) dissolved in 8% alcohol and in Group V with 0.3 ml. of 70% alcohol on the first, fourth and seventh day. The left knee of each animal in all five groups was simultaneously injected with 0.3 ml. of physiological saline as a control.

Studies at the time of sacrifice

One animal from each group was sacrificed at one, six, twelve and twenty-four weeks after the last intra-articular injection. Both knees were removed without opening the joint or disturbing the surrounding soft tissues by dividing the tibia and the femur through the midshaft. Roentgenograms of the knees were done after they were rapidly deep frozen. Coronal sections were cut from each specimen and stained with haematoxylin-eosin, periodic acid schiff (PAS) — alcian blue, Safranin-O and Martius red-crystal scarlet and celestine blue (MSB).

Histochemical Stains

Alcian blue and Safranin-O which are cationic dyes bind to the polyanions of the protein polysaccharides namely chondroitin sulphate and Keratan sulphate (Meachim and Stockwell, 1973) and are useful to demonstrate depletion of glycosaminoglycans of the cartilage matrix which is the earliest change in experimental osteoarthritis. Alcian blue is superior to most other cationic dyes and shows intense blue staining in zones 2 and 3 of normal articular cartilage. When PAS is added, Keratan sulphate which is remote from the cartilage cells and chondroitin sulphate which is in the vicinity of the cells can be demonstrated by differential staining.

Safranin-O which is a cationic orthochromatic dye, binds to the polyanions of the protein polysaccharides (Rosenberg, 1971) giving a uniform red staining in zones 1, 2 and 3 of the normal cartilage.

The MSB stain contrasts the bone which stains red with blue osteocyte nuclei against the unclacified cartilage which stains blue with dark brown chondrocyte nuclei (Bentley, 1971) and is useful to demonstrate the viability of the cartilage cells.

RESULTS

The control left knees and the right knees injected with acetylsalicylic acid and prostaglandin B_2 did not reveal any roentgenographic changes. However knees injected with papain, indomethacin and alcohol showed narrowing of the joint space, subchondral sclerosis and osteophytic lipping in varying degrees twenty-four weeks after the last intra-articular injection. These changes were more marked after papain injections (Fig. 1).

Histochemical staining of the control knees and of the knees injected with acetylsalicylic acid and prostaglandin B^2 showed no alteration in the staining characteristics of the articular cartilage, or of the cartilage cells throughout the period of the study. In contrast intra-articular injections of papain, indomethacin and alcohol produced quite marked cartilage changes.

One week after papain injection, staining with Safranin-O and PAS-alcian blue were considerably diminished and in focal areas the articular cartilage showed fibrillation, clefting and erosion (Fig. 2a). The changes were more advanced at six, twelve and twenty-four weeks after papain injections (Fig. 2b).

Changes produced after intra-articular injections of indomethacin were similar to those seen after papain injections (Fig. 3).

The alcohol injected knees revealed extensive cartilage cell death, fibrillation and decreased staining to PAS-alcian blue and Safranin-O in zones 1, 2 and 3 from six weeks onwards (Fig. 4).

Many of the specimens in these three groups showed proliferation of cartilage cells from deeper layers with intense pericellular alcian blue staining, indicative of synthesis of new chondroitin sulphate in an attempt at repair (Fig. 5).

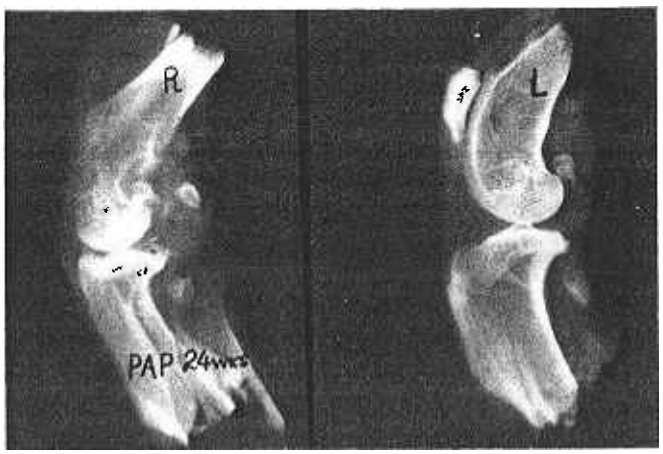

Fig. 1. Lateral roentgenograms of control left knee and papain injected right knee twenty-four weeks after the last intra-articular injection. Note the marked narrowing of the joint space, subchondral sclerosis and lipping of the tibial articular margins on the right side.

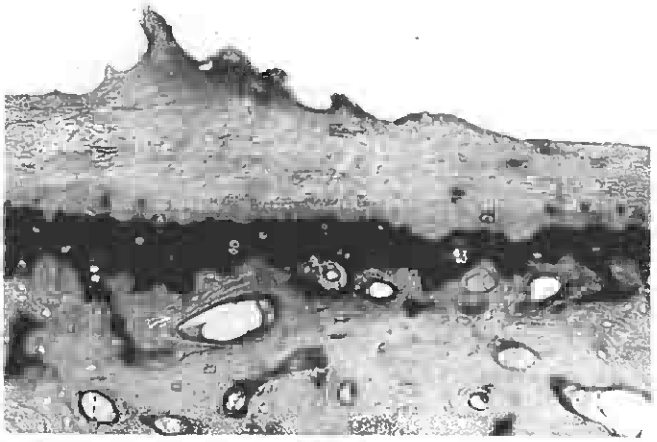

Fig. 2a. Section through a right knee one week after the last intra-articular injection of papain. Note the irregularity of the articular cartilage and complete absence of cartilage cells and staining of the cartilage matrix except in the zone of calcified cartilage (Safranin-O X125).

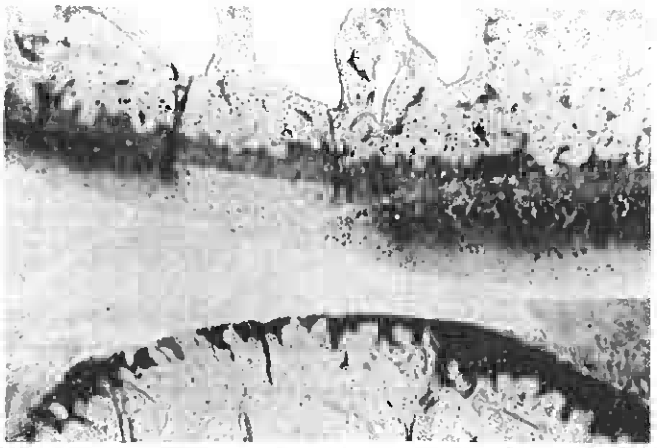

Fig. 2b. Section through a right knee twenty-four weeks after the last intra-articular injection of papain. Note that the articular cartilage is eroded to the level of the subchondral bone (below) while the opposing articular surface (above) shows acellularity, loss of staining of zones 1, 2 and 3 and superficial erosion of the cartilage (Safranin-O X40).

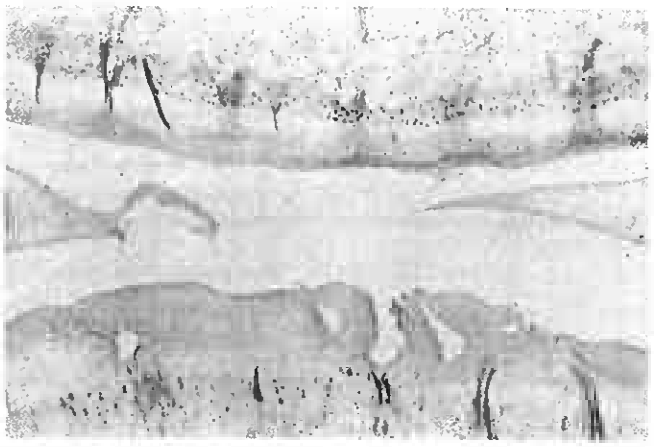

Fig. 3. Section through a right knee twenty-four weeks after the last intra-articular injection of indomethacin. Note the flaking and clefting of the articular cartilage (below) and loss of staining of the matrix and acellularity in zones 1, 2 and 3 of the cartilage (both above and below) (PAS-alcian blue X40).

Fig. 4. Section through a right knee twenty-four weeks after the last intra-articular injection of 70% alcohol. Note marked fibrillation and flaking of the articular cartilage and complete acellularity in zones 1, 2 and 3 (Martius red-crystal scarlet and celestine blue X250).

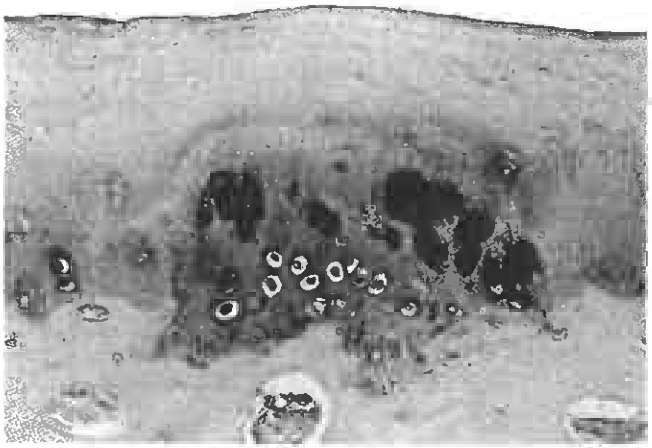

Fig. 5. Section through a right knee six weeks after the last intra-articular injection of 70% alcohol. Note absence of staining and cells in zones 1, 2 and 3 of the articular cartilage and proliferation of a cluster of cartilage cells from the deeper layers (in the centre) with intense pericellular staining of the matrix (PAS-alcian blue X250).

DISCUSSION

This study confirms the observations of previous workers (Sweet and Solomon, 1968; Bentley, 1971, 1975) that intra-articular injections of papain produce progressive articular cartilage damage which can be demonstrated roentgenographically and by histochemical studies. Papain which is a proteolytic enzyme breaks the bond between the protein core and the glycosaminoglycans in a manner comparable to that produced by cathepsin D (Bentley, 1975). It also produces death of the chondrocytes in the superficial zones of the articular cartilage (Bentley, 1975). Similar progressive changes were observed after intra-articular injections of indomethacin and alcohol. Absolute alcohol is known to have a direct toxic effect on the cartilage matrix and its cellular elements. However the exact mode of action of indomethacin in producing the cartilage changes is not known. Five percent alcohol used as a solvent for indomethacin could not have been responsible for the articular cartilage changes, as similar changes were not observed with prostaglandin B_2 which was dissolved in eight percent alcohol. Most anti-inflammatory drugs are known to protect the articular cartilage in rheumatoid arthritis by suppressing the inflammation through inhibition of the prostaglandin synthetase systems (Vane, 1971, 1972; Takeguchi and Sih, 1972; Willis et al, 1972; Moncada et al, 1973). However recent in vitro culture studies on full-depth samples of aged articular cartilage from femoral heads removed following subcapital fracture have shown that sodium salicylate, indomethacin, ibuprofen and hydrocortisone inhibited sulphated glycosaminoglycan synthetisis (McKenzie et al, 1976). Arthropathy following intra-articular injections of corticosteroids has been reported clinically on roentgenographic evidence (Chandler and Wright, 1958) and in rabbits by the administration of steroids (Salter et al, 1967). We were not able to find any report of the effect of intra-articular injections of acetylsalicylic acid or indomethacin in experimental animal. Although sodium salicylate in varying concentrations produces depletion of glycosaminoglycans of the cartilage strips in tissue culture (McKenzie et al, 1976) intra-articular injections of acetylsalicylic acid in rabbit knees in our series did not show any histochemical changes in the articular cartilage. It is possible that measuring the uptake of $^{35}SO_4$ in the vitro studies is a more sensitive method of assessment of glycosaminoglycan synthesis than the histochemical methods used in the present study.

Although other authors have noted inhibition of cartilage synthesis in embryonic chicken cartilage in culture medium by prostaglandin B_1 (Eisenbarth and Lebovitz, 1974; Kirkpatrick and Gardner, 1976) and cartilage degradation and synovitis after intra-articular injections of prostaglandin E_1 in rabbit knees (Teitz and Chrisman, 1975), we have not been able to confirm these observations by intra-articular injections of prostaglandin B_2 in rabbit knees. It is possible that the effective concentration of PGB_2 used in our series was not as high as that attained by the previous workers.

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