Experience with isoxicam and catabolin

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1 Synovial cells produce a protein factor, catabolin, that induces chondrocytes to resorb their own matrix with the release of glycosaminoglycan (GAG).

2 Studies were performed to determine the effect of isoxicam, a new NSAID, on GAG production in a catabolin system utilizing tissue culture of bovine nasal septum in a medium containing pooled synovial tissue from young pigs.

3 Using fresh medium containing no synovial tissue, the release of GAG by inherent cartilage breakdown was 49.7% after 8 days culture. GAG release at 8 days increased to 83.6% by the addition of synovial cells to the culture medium, due to the action of catabolin.

4 The incorporation of isoxicam, at concentrations from 5–30 $\mu\text{g ml}^{-1}$, in the porcine synovial culture medium significantly reduced the GAG release to 70.9%.

5 Isoxicam at 50 $\mu\text{g ml}^{-1}$ added to fresh medium without synovial tissue had no effect on cartilage breakdown or GAG production.

6 The results of these experiments show that isoxicam is unlikely to impair cartilage metabolism and may even decrease cartilage resorption.

Keywords isoxicam catabolin glycosaminoglycan tissue culture cartilage

Introduction

Mechanisms by which connective tissue is catabolised during inflammation are obscure. That chondrocytes may resorb their surrounding matrix was first demonstrated by Fell & Mellanby (1952) who showed that, in cartilage culture, vitamin A stimulates the release of an acid protease. Later, when cartilage was co-cultured with synovial tissue in the same vessel but not in contact, degradation was much greater than when cartilage was cultured on its own (Fell & Jubb, 1977). Subsequent work by Dingle *et al.* (1979) indicated that synovial cells produce a protein factor of 15,000–20,000 daltons which induces chondrocytes to resorb their own matrix with the release of glycosaminoglycan (GAG). This factor was named 'catabolin' and provided an alternative hypothesis to explain the cartilage destruction seen in arthritis.

Of the wide range of drugs used in the treatment of arthritis, only a few have as yet been tested on the porcine catabolin system described by Dingle *et al.* (1979). Aspirin, clobuzarit (Closic) indomethacin and gold at therapeutic concentrations have all been shown to have no effect on catabolin production in the porcine system or any direct action on cartilage resorption (Sheppeard *et al.*, 1982; Dingle, 1981). Similar results were observed when human rheumatoid synovium was co-cultured with cartilage in the presence of gold and indomethacin (Steinberg *et al.*, 1979).

The action of catabolin on cartilage may be enhanced by some drugs such as penicillamine while others such as chloroquine have an inhibitory effect (Sheppeard *et al.*, 1982). Corticosteroids in low doses, while inhibiting the produc-

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tion of catabolin by synovial cells, have no effect on its stimulation of chondrocytes, even in high doses (Saklatvala, 1981).

Oxicams are a new class of non-steroidal anti-inflammatory drugs (NSAIDs) which provide a continuous activity over 24 h due to their long plasma life. Single-daily-dose therapy compares favourably with drugs taken in more frequent doses, e.g., naproxen, indomethacin. Isoxicam has a higher solubility than other oxicams, a wide therapeutic range and good patient tolerance. Peak concentrations after a single dose of 200 mg are in the range of $10 \mu\text{g ml}^{-1}$ (Kölle & Vollmer, 1986) whereas the steady state concentrations after dosing of 200 mg daily vary between 25 and $40 \mu\text{g ml}^{-1}$.

Methods

Pooled synovial tissues from the foretrotters of young pigs are washed in a balanced salt solution then minced with scissors after removal of most of the fluid. Aliquots of 0.05 ml are measured with a plastic Mantoux syringe from which the needle mount is cut with a hot scalpel blade. The cells are cultured in Dulbeccos modification of Eagle's medium (Flow laboratories) with HEPES buffer supplemented with 5% foetal calf serum, penicillin, streptomycin and amphotericin and incubating in air. The medium is harvested at days 3 and 6 and pooled with 5 ml of fresh medium to make a total of 9 ml for cartilage culture. Hydrocortisone succinate added at $1 \mu\text{g ml}^{-1}$ inhibits inherent cartilage breakdown but not the action of catabolin.

Discs of bovine nasal septum, 4 mm diameter by 1.5 mm thick, are cultured in this medium for

4 days and then a further 4 days after medium change. The cartilage disc remaining after culture is digested with papain in 5 ml of neutral phosphate buffer containing EDTA and *N*-acetyl cysteine. The amount of papain is adjusted to give complete dissolution after 2 h at 60°C . The cartilage culture harvested medium, nominally 1 ml, is diluted with 4 ml of distilled water.

Glycosaminoglycans (GAG) are measured spectrophotometrically at 527 nm with a stable solution of dimethylmethylene blue (Serva) as described by Farndale *et al.* (1982), using shark chondroitin sulphate (Sigma) as the standard. Catabolin activity is expressed as the percentage of GAG released into the medium.

Isoxicam at concentrations of 5, 15 and $30 \mu\text{g ml}^{-1}$ were incorporated in the synovial culture medium. Isoxicam at $50 \mu\text{g ml}^{-1}$ was added to fresh medium containing hydrocortisone to determine its direct effect on inherent cartilage breakdown.

Results

Cartilage degradation measured as a percentage GAG released in experiments with fresh medium and synovial culture medium with and without isoxicam, are combined in Table 1. During tissue culture, cartilage normally releases GAG, this inherent cartilage breakdown after 8 days culture in fresh medium was 46–53%. The action of catabolin in the synovial culture medium, stimulates living chondrocytes to resorb their own matrix and increase the GAG release to 66–85% over the same period of time. At all concentrations, isoxicam partially reduced

Table 1 Percentage of GAG released (rounded to the nearest whole number) in three separate experiments with isoxicam (I) in the synovial culture medium.

		% GAG released				<i>F</i> vs <i>I</i>		<i>Student's t</i> -test <i>I</i> vs <i>C</i>			
	<i>Exp</i>	<i>Isoxicam</i> ($\mu\text{g ml}^{-1}$)	<i>F</i>	<i>I</i>	<i>C</i>	<i>t</i>	<i>df</i>	<i>P</i>	<i>t</i>	<i>df</i>	<i>P</i>
<i>A</i>	1	5	14	18	36	2.10	30	*	9.97	30	**
	2	10	18	21	29	2.99	23	**	3.98	23	**
	2	30	18	22	29	2.62	23	*	3.47	23	**
	3	7–30	18	35	42	4.98	38	**	2.16	37	*
<i>B</i>	1	5	53	68	85	6.9	30	**	10.83	30	**
	2	10	46	55	66	2.47	23	*	3.71	23	**
	2	30	46	57	66	2.98	23	**	2.58	23	*
	3	7–30	50	71	84	5.64	38	**	3.62	38	**

A = 4 days of culture, *B* = 8 days of culture, *F* = fresh medium, *I* = isoxicam, *C* = catabolin.

* $P < 0.01$, ** $P < 0.001$, *df* = degrees of freedom.

the catabolin induced GAG release but no dosage succeeded in reducing this release to the level of inherent cartilage degradation as shown in Figure 1. Experiment 3 did not show a dose relationship between isoxicam and percentage GAG released. The results were 69.9%, 70.9% and 71.9% for 7, 15 and 30 $\mu\text{g ml}^{-1}$ respectively after 8 days of culture and were not significantly different; consequently they have been combined in Table 1.

When cartilage discs were cultured in fresh medium containing isoxicam at 50 $\mu\text{g ml}^{-1}$, the inherent breakdown was unchanged.

Discussion

In rheumatoid arthritis, the concept of cartilage breakdown being solely due to the direct eroding action of the rheumatoid pannus is no longer tenable. Damaged or inflamed synovial tissue stimulates catabolin production which induces chondrocyte-mediated matrix breakdown (Dingle, 1979). In osteoarthritis, where inflammatory activity is minimal, catabolin may play the major role in cartilage degradation. Further experiments are required to confirm the presence of catabolin-like substances in the pathology of rheumatoid or osteoarthritis. However, assessing NSAIDs for cartilage-protecting properties appears a worthwhile goal.

The inclusion of isoxicam in the culture medium did not completely abolish the production of catabolin by synovial cells, but significantly reduced the amount. The relationship between these findings and the *in vivo* situation in which there is continual replacement of nutrients and drug is open to speculation. There may be a compensatory, increased synthesis of catabolin due to the ready availability of nutrients. Alternatively, the constant renewal of isoxicam at therapeutic levels may further retard the production of catabolin. Isoxicam did not increase the rate of inherent cartilage degradation, suggesting that it has no inhibitory effect on proteoglycan synthesis.

The inherent cartilage breakdown in these experiments was higher than obtained previously by Sheppard *et al.* (1982), but so was the catabolin-induced breakdown. This inherent breakdown shows considerable variation between animals and can be reduced by the inclusion of 1 $\mu\text{g ml}^{-1}$ hydrocortisone in the cartilage culture medium (Saklatvala, 1981). This observation suggests that catabolin-like molecules may be produced by chondrocytes themselves.

Figure 1 Percentage (mean $\pm$ s.e. mean) of GAG released from culture for 4 and 8 days from bovine nasal cartilage, (a) in fresh medium, (b) in spent medium from porcine synovial tissue culture and (c) in the same medium to which isoxicam at 5–30 $\mu\text{g ml}^{-1}$ had been added.

The catabolin culture system provides a means for the testing of new anti-arthritis drugs and the development of more effective analogues. In addition, any deleterious effect on the behaviour of chondrocytes may be assessed. For some NSAIDs, particularly indomethacin, in osteoarthritis of the hip, there is considerable incriminating evidence in this regard (Jubb, 1984). Our experiments show that isoxicam's anti-inflammatory action is unlikely to impair cartilage metabolism and may even decrease cartilage resorption.

Addendum

Recent experiments have shown that isoxicam at 50 $\mu\text{g ml}^{-1}$ partially inhibited the action of pre-formed catabolin on bovine nasal cartilage, reducing the percentage of GAG released from 80 to 64% ($t = 3.7$, d.f. 17, $P < 0.01$). Isoxicam at 5 $\mu\text{g ml}^{-1}$ had no effect in this experiment.

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