Effects of antirheumatoid drugs on the production and action of porcine catabolin

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SUMMARY We report the effects of some common antirheumatic drugs on the production of catabolin from synovium and on its action on cartilage. A method is described to generate reproducible amounts of catabolin from synovial mince. Aspirin, Clozic (ICI 55 897), and gold were without effect on the catabolin system. Penicillamine at high doses enhanced the action of catabolin, while chloroquine inhibited catabolin's effect on cartilage. Prednisolone inhibited the production of catabolin without affecting its action. This inhibition was produced by very low doses of prednisolone (25 ng/ml) and was dose-dependent.

Synovial tissue in culture produces a factor, catabolin, which causes living cartilage in vitro to resorb its matrix.¹⁻⁴ It is assayed by its ability to cause loss of glycosaminoglycan from cartilage in culture. Medium conditioned by pig synovium contains only one major component active in inducing matrix loss in bovine nasal cartilage, a protein of molecular weight 17 000–18 000 and an isoelectric point of pH 4.6.⁵

The experiments shown below were undertaken to investigate the effects of some common antirheumatoid drugs on the production of catabolin from synovium and on its action on cartilage in culture. It was necessary to develop a means of generating reproducible quantities of catabolin from synovial mince so that the amount of catabolin produced from tissue treated with the various drugs could confidently be compared with that from the control cultures.

Materials and methods

PRODUCTION OF CATABOLIN

The joint capsule was dissected from the third and fourth metacarpophalangeal joints of freshly killed young pigs and the synovium carefully removed. Pooled synovial tissue was minced, washed, and centrifuged at 600 g for 10 minutes to produce a compact mass of tissue, which was aliquoted by filling a sterile plastic mould. The measured volume of mince was spread thinly on to filter paper supported by a stainless steel grid and cultured in 2 ml of Dulbecco's modified Eagle's medium supplemented with 60 µg

per ml glutamine, penicillin/streptomycin (200 IU per ml and 200 µg per ml respectively), and 5% fetal calf serum. The cultures were incubated at 37°C in an atmosphere of 5% CO₂, 20% O₂, and 75% N₂ for 6 days, the medium being changed at day 3. Samples of mince which were killed by freezing and thawing were cultured to serve as controls. Medium harvested at day 3 and day 6 from each synovial mince was pooled and diluted with 5 ml of culture medium to yield 9 ml for assay of catabolin activity.

The following drugs were added to the cultures of synovial mince: aspirin, Clozic (ICI 55 897), gold (sodium aurothiomalate), prednisolone, D-penicillamine, and chloroquine. The drugs were added at various concentrations, including those reported to be found in the plasma and tissues of patients undergoing drug therapy (Table 1). Between 3 and 6 cultures of synovial mince were used in each experimental group.

ASSAY OF CATABOLIN

Culture media conditioned by the synovial mince were added to discs of cartilage prepared from bovine nasal septum, which were then maintained for 8 days.³ Discs which had been killed by freezing and thawing were included in each experiment. At the end of the culture period the discs were digested with papain, and the glycosaminoglycan present in the digests and the medium was measured spectrophotometrically with dimethylmethylene blue.⁵ Catabolin activity was expressed as:

$$\frac{\text{chondroitin sulphate released into medium}}{\text{total chondroitin sulphate from each disc}} \times 100.$$

Table 1 The concentrations of antirheumatoid drugs used in the experiments and their relationship to therapeutic levels

Drug	Concentration used	Pharmacological Rationale	References
Aspirin	250 µg/ml	Plasma therapeutic range 200–300 µg/ml	Bayles ⁶
Clozic (ICI 55 897)	140 µg/ml	Plasma therapeutic range 100–300 µg/ml	Billingham personal communication
Gold (Myocrisin)	10 µg/ml 100 µg/ml	Peak plasma level Tissue level	Jessop and Johns ⁷ Grahame <i>et al.</i> ⁸
Prednisolone (Codesol)	2.5 ng/ml 25 ng/ml 250 ng/ml 2.5 µg/ml	10 ⁻¹ × peak plasma level Equivalent to physiological cortisol level Peak plasma following 10 mg dose 10 × peak plasma level	Pickup ⁹
D-penicillamine (Distamine)	50 µg/ml 100 µg/ml 250 µg/ml 500 µg/ml	Peak plasma level following 1 g dose Plasma concentrations on 1 g daily 5 × peak plasma level 10 × peak plasma level	Gibbs and Walshe ¹⁰ Mowat ¹¹
Chloroquine phosphate (Avloclor)	200 ng/ml 400 ng/ml 4 µg/ml 40 µg/ml	Peak plasma level following 500 mg dose 10 ⁻² × tissue level 10 ⁻¹ × tissue level Tissue level (200 × peak plasma level)	Goodman and Gilman ¹²

SI conversion: µg/ml=mg/l; ng/ml × 10³=mg/l.

As the drugs added to the synovial mince were still present during the bioassay with the bovine nasal cartilage, their direct effects on the action of catabolin on cartilage were assessed as follows. Partially purified catabolin³ was added to cultures of bovine nasal cartilage discs in the presence of prednisolone, D-penicillamine, or chloroquine. The action of D-penicillamine alone on cartilage was examined by adding the drug to cultures of both living and killed discs.

Chondroitin sulphate release was measured as described above.

EFFECT OF CHLOROQUINE ON UPTAKE OF ³H-GLYCINE BY BOVINE NASAL CARTILAGE

The effect of chloroquine on protein synthesis by the cartilage was studied. Groups of discs (3 per group) were cultured for 8 days as before in the presence of chloroquine at concentrations of 4 µg/ml, 40 µg/ml and 400 µg/ml (SI conversion: µg/ml=mg/l). At day 1 of culture some of the discs were incubated for 4 hours in culture medium containing 5 µCi per ml ³H-glycine (Amersham, Bucks) and washed 3 times in medium containing 1 mg/ml non-radioactive glycine. At the end of the culture period the discs were left overnight in a mixture of ethanol and 1 mg/ml glycine, washed again 3 times in ethanol-glycine and digested with papain as described. Sam-

ples of this digest were taken for liquid scintillation counting in a mixture of toluene scintillator and picoflour 30 (Packard) 50:50 v/v.

The procedure was repeated on day 4, day 6, and day 8.

Results

Medium from the cultures of synovial mince caused marked release of chondroitin sulphate from discs of bovine nasal cartilage.

By using a mould to measure out synovial mince for culture we obtained reproducible wet weights of tissue aseptically (58.2 mg wet weight, SEM 1.5%, n=8). The variation in release of chondroitin sulphate produced by medium from the synovial cultures was considered to show acceptable reproducibility for this type of bioassay (52%, SEM 3%, n=8) in 8 days. Dead cartilage cultured under the same conditions produced 22.1% (SEM 1%, n=8) release of chondroitin sulphate, and control cultures of living cartilage in medium alone resulted in 19.4% (SEM 1.1%, n=8) release in 8 days. There was no significant difference between chondroitin sulphate release from bovine nasal cartilage cultured in the presence of medium from control synovial mince and that from synovial mince treated with either aspirin, Clozic, or gold (Fig. 1) at various doses.

Fig. 1 Cumulative release of chondroitin sulphate from bovine nasal cartilage cultured for 4 and 8 days in the presence of spent medium from cultures of untreated pig synovial mince, and of medium from mince cultured for 6 days in the presence of gold, aspirin, and Clozic.

The addition of prednisolone to cultures of synovial mince caused medium from these cultures to elicit less breakdown of bovine nasal cartilage than was produced by spent medium from untreated synovium. This effect was dose-dependent, a highly significant ($p \leq 0.001$) decrease in chondroitin sulphate release being produced by medium from synovial cultures which had been maintained in the presence of 25 ng/ml prednisolone (Fig. 2).

Fig. 2 Cumulative release of chondroitin sulphate from discs of bovine nasal cartilage cultured for 4 days or 8 days in the presence of medium from cultures of untreated pig synovium, and of medium from mince maintained in the presence of increasing doses of prednisolone.

When prednisolone (250 ng/ml) was added to cultures of bovine nasal cartilage in the presence of partially purified catabolin no inhibition of degradation was produced (Fig. 3).

Medium from cultures of synovial mince maintained in the presence of 500µg/ml D-penicillamine

Fig. 3 The addition of prednisolone has no significant effect upon the release of chondroitin sulphate by cartilage discs cultured in the presence of partially purified catabolin.

Fig. 4 Cumulative release of chondroitin sulphate from discs of bovine nasal cartilage cultured for 4 days and 8 days in the presence of medium from untreated pig synovium, and of medium from mince maintained in the presence of increasing doses of D-penicillamine.

Fig. 5 The cumulative release of chondroitin sulphate from discs of bovine nasal cartilage maintained in vitro in the presence of partially purified catabolin is enhanced by the addition of D-penicillamine.

Fig. 6 Cumulative release of chondroitin sulphate from discs of bovine nasal cartilage cultured for 4 days or 8 days in the presence of medium from untreated pig synovium, and of medium from mince maintained in the presence of chloroquine.

caused more breakdown of the cartilage discs than did the spent medium from untreated mince. The addition of 50 µg/ml D-penicillamine to the synovial cultures had no effect (Fig. 4). When this drug was added to cartilage cultures in addition to partially purified catabolin, enhancement of the catabolin-induced chondroitin sulphate release occurred (Fig. 5) at doses of 250 µg/ml and 500 µg/ml D-penicillamine ($p \leq 0.01$).

D-penicillamine alone had no effect on breakdown of either living or dead cartilage.

Fig. 7 The addition of chloroquine to cultures of bovine nasal cartilage discs reduces the release of chondroitin sulphate produced by partially purified catabolin.

Fig. 8 Uptake of ³H-glycine by cartilage discs in vitro is unimpaired by the addition of chloroquine (0.4–40 µg/ml) to the culture medium.

After the addition of chloroquine to cultures of synovial mince, the resulting medium caused less breakdown of cartilage than did spent medium from untreated mince (Fig. 6). This effect was significant ($p < 0.01$) at dose levels of 40 µg/ml chloroquine. The addition of chloroquine to the cartilage culture in the presence of partially purified catabolin also produced inhibition of catabolin-induced breakdown, the inhibition being dose-dependent (Fig. 7), a significant inhibition was produced by a dose of 40 µg/ml ($p = 0.01$ – 0.05).

Incorporation of ³H-glycine by the cartilage discs in culture was unimpaired by chloroquine at doses of 400 ng/ml (Fig. 8).

Discussion

It proved possible to standardise the culture conditions for catabolin production from synovial mince, so that the resulting release of glycosaminoglycan from the discs of nasal cartilage used in the assay was in close agreement, permitting the analysis of experimental modification of catabolin production.

Aspirin, Clozic, and gold were without effect on either the production of catabolin or its action on cartilage. Clozic has now been withdrawn by ICI and will not become available on prescription.

Prednisolone inhibited production of catabolin but did not interfere with its action on cartilage. This inhibition occurred at a concentration of 25 ng/ml

prednisolone, a level well below those achieved when the drug is used for its anti-inflammatory or immunosuppressive effects.¹³ Other corticosteroids when added to human synovium in culture have been shown to exert a similar inhibition of cartilage resorption.¹⁴ Obviously caution must be exercised in extrapolating from organ culture experiments with animal tissues to chemotherapy of the human disease, but it is quite possible that catabolin production is suppressed in patients undergoing prednisolone therapy.

It is not known to what extent, if any, catabolin is stored by tissues, but any such residual catabolin would not be inhibited in its action on cartilage by prednisolone. The activity of catabolin on cartilage was not suppressed even by very high doses of the drug, levels at which the release of lysosomal enzymes from the cartilage itself would be substantially inhibited.¹³ The mechanism of cartilage resorption remains obscure.

Penicillamine when used at high dose levels, enhanced the action of catabolin. Although the drug has an extremely long biological half-life and has been reported to be concentrated in joint structures,^{15,16} the levels effective in enhancing breakdown of cartilage in these experiments, namely, 250 µg/ml and 500 µg/ml, are unlikely to be achieved in clinical practice.^{10,11} The drug alone had no effects on the cartilage discs but required the presence of catabolin to bring about matrix loss.

Chloroquine inhibited the action of catabolin on cartilage. Since it is catabolin's effect on cartilage which forms the basis for its assay, it is not possible to comment from these results about chloroquine's action on catabolin production. The dose levels required to produce the inhibition of activity were very high, but in animal studies, tissue concentrations of 200 to 700 times the plasma level of chloroquine have been measured.¹² The concentrations effective in the experiments reported here could be reached in cells which accumulate the drug.¹⁷

Glycine incorporation by the cartilage discs cultured in the presence of chloroquine did not differ from that of the controls, implying that the drug is not toxic to chondrocytes.

This study is a first step in trying to understand whether the catabolin system can be modified by commonly used antirheumatic drugs. The increased cartilage destruction brought about by adding D-penicillamine is only of pharmacological interest as the effective doses are too high to be achieved clinically. Of the 2 drugs shown to cause a net sparing of cartilage, prednisolone caused a marked inhibition of the production of catabolin, while chloroquine exerted its effect by suppressing the action of catabolin.

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