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. 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.

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. 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 dimethylmethylen blue.

Catabolin activity was expressed as:

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

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

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration used</th>
<th>Pharmacological Rationale</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>250 μg/ml</td>
<td>Plasma therapeutic range 200–300 μg/ml</td>
<td>Baylesa</td>
</tr>
<tr>
<td>Clozic (ICI 55 897)</td>
<td>140 μg/ml</td>
<td>Plasma therapeutic range 100–300 μg/ml</td>
<td>Billingham personal communication</td>
</tr>
<tr>
<td>Gold (Myocrisin)</td>
<td>10 μg/ml</td>
<td>Peak plasma level</td>
<td>Jessop and Johns'Grahame et al.'s</td>
</tr>
<tr>
<td>Prednisolone (Codesol)</td>
<td>2-5 ng/ml</td>
<td>Equivalent to physiological cortis level</td>
<td>Pickup®</td>
</tr>
<tr>
<td></td>
<td>25 ng/ml</td>
<td>Peak plasma following 10 mg dose</td>
<td></td>
</tr>
<tr>
<td></td>
<td>250 ng/ml</td>
<td>10 × peak plasma level</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-5 μg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-penicillamine</td>
<td>50 μg/ml</td>
<td>Peak plasma level following 1 g dose</td>
<td>Gibbs and Walshe&quot;</td>
</tr>
<tr>
<td>(Distamine)</td>
<td>100 μg/ml</td>
<td>Plasma concentrations on 1 g daily</td>
<td>Mowat&quot;</td>
</tr>
<tr>
<td></td>
<td>250 μg/ml</td>
<td>5 × peak plasma level</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500 μg/ml</td>
<td>10 × peak plasma level</td>
<td></td>
</tr>
<tr>
<td>Chloroquine phosphate</td>
<td>200 ng/ml</td>
<td>Peak plasma level following 500 mg dose</td>
<td>Goodman and Gilman&quot;</td>
</tr>
<tr>
<td>(Avloclor)</td>
<td>400 ng/ml</td>
<td>10^−9 × tissue level</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 μg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 μg/ml</td>
<td>Tissue level (200 × peak plasma level)</td>
<td></td>
</tr>
</tbody>
</table>

SI conversion: μg/ml=mg/l; ng/ml×10^9=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. Samples of this digest were taken for liquid scintillation counting in a mixture of toluene scintillator and pico-flour 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.
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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).

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 500 \mu g/ml D-penicillamine...
EFFECTS OF D-PENICILLAMINE UPON THE PRODUCTION OF CATABOLIN FROM PIG SYNOVIAL MINCE AND ITS ACTION ON BNC

![Graph of Cumulative Release of Chondroitin Sulphate (%)](image)

- **UNTREATED SYNOVIAL CULTURES**
- **SYNOVIAL CULTURES + D-PENICILLAMINE 500 μg/ml**
- **SYNOVIAL CULTURES + D-PENICILLAMINE 50 μg/ml**
- **MEDIUM ALONE**

(P = peak plasma level achieved therapeutically)

![Graph of Cumulative Release of Chondroitin Sulphate (%)](image)

- **UNTREATED SYNOVIAL CULTURES**
- **SYNOVIAL CULTURES + CHLOROQUINE 40 μg/ml**
- **SYNOVIAL CULTURES + CHLOROQUINE 200 μg/ml**
- **MEDIUM ALONE**

(P = peak plasma level achieved therapeutically)

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. 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 = <<0.01).

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

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EFFECTS OF CHLOROQUINE UPON THE PRODUCTION OF CATABOLIN BY PIG SYNOVIAL MINCE AND ITS ACTION ON BNC

![Graph of Cumulative Release of Chondroitin Sulphate (%)](image)

- **UNTREATED SYNOVIAL CULTURES**
- **SYNOVIAL CULTURES + CHLOROQUINE 40 μg/ml**
- **SYNOVIAL CULTURES + CHLOROQUINE 200 μg/ml**
- **MEDIUM ALONE**

(P = peak plasma level achieved therapeutically)

Fig. 7 The addition of chloroquine to cultures of bovine nasal cartilage discs reduces the release of chondroitin sulphate produced by partially purified catabolin.
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**Figure 8** Uptake of 

1. 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 

2. Prednisolone, a level well below those achieved when the drug is used for its anti-inflammatory or immunosuppressive effects. Other corticosteroids when added to human synovium in culture have been shown to exert a similar inhibition of cartilage resorption. 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. The mechanism of cartilage resorption remains obscure.

3. 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, 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. 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. The concentrations effective in the experiments reported here could be reached in cells which accumulate the drug.

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.
References

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