Corticosteroid treatment of experimental arthritis retards cartilage destruction as determined by histology and serum COMP

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Objective. To examine if changes in serum cartilage oligomeric matrix protein (COMP) correlate with the development of cartilage damage, as measured by histological grading, in corticosteroid-treated animals with collagen-induced arthritis (CIA).

Methods. DA rats with established CIA were treated with corticosteroids (betamethasone, 0.1 mg/kg body weight) or placebo (saline) intraperitoneally once daily after reaching an arthritis score exceeding 1. The treatment continued throughout the study. Arthritis progression was monitored by clinical scoring of paws, serial measurements of serum COMP and fibrinogen, and histological grading of paws.

Results. Corticosteroid treatment reduced clinical signs of arthritis compared with placebo (arthritis score reduced, \(P < 0.01\) at day 25). Corticosteroid treatment also reduced fibrinogen levels compared with placebo (\(P < 0.01\)). The morphological changes in the joint were less severe in the corticosteroid-treated animals (median cartilage score 4 in the placebo group, 0 in the corticosteroid-treated group; \(P < 0.01\)). The levels of COMP remained unchanged during treatment in the corticosteroid-treated arthritic animals, whereas an increase in levels of COMP was observed in rats treated with placebo (\(P < 0.01\)). There was a correlation between serum COMP and the extent of cartilage destruction at day 25 after immunization (\(r = 0.77, P < 0.001\)).

Conclusions. Corticosteroids given therapeutically to arthritic rats diminish joint destruction histologically, and stable serum COMP levels reflect this effect.

Key words: Collagen-induced arthritis, Cartilage oligomeric matrix protein (COMP), Fibrinogen, Corticosteroids.

Prevention of tissue destruction is one of the aims of modern anti-rheumatic therapy. Evaluation of the tissue-protective properties of drugs is, however, hampered by the fact that radiography, the gold standard for the quantification of joint destruction, is insensitive. A time span of at least 6 months is needed to detect differences between groups treated in different ways [1]. Preclinical evaluation of anti-rheumatic drugs is often performed in animal disease models, and collagen-induced arthritis (CIA) is one of the most frequently used of these. CIA presents with a destructive polyarthritis that has many clinical [2] and radiological similarities to rheumatoid arthritis (RA). In order to improve the early evaluation of anti-rheumatic drugs for their tissue-protective capacity, there is an obvious need for biomarkers which can be used both in animal models and in human arthritis, and which rapidly mirror changes in cartilage turnover.

Cartilage oligomeric matrix protein (COMP) [3] is a candidate biomarker for the monitoring of cartilage destruction. It has been shown to reflect cartilage destruction during the course of disease in both human arthritis [4] and in experimental arthritis [5], as well as in therapeutic intervention studies [6, 7].

Several disease-modifying anti-rheumatic drugs have been shown to retard joint tissue destruction in RA [8]. Corticosteroids have also been claimed to have these effects, but this opinion is controversial [9].

The aim of this study was to investigate the extent to which corticosteroids can retard cartilage destruction as determined by histology in CIA [10], and to evaluate serum COMP for monitoring this putative cartilage-protective effect.

Materials and methods

Animals

Female DA rats aged 3.5–4 months at the initiation of the experiments were used. The health of the animals was monitored according to the guidelines of the Swedish Veterinary Board, and they were found to be free of pathogens. The rats were originally obtained from Zentralinstitut für Versuchs-tierzucht, Hannover, Germany, and were bred, kept and used at the Karolinska Hospital, Stockholm, Sweden. They were maintained in a climate-controlled environment with 12 h light/12 h dark cycles with free access to rodent food and water. The ethics board for animal experiments in Stockholm-North approved all the animal procedures.
Corticosteroid treatment of experimental arthritis retards cartilage destruction

Induction and clinical monitoring of CIA

Collagen II was prepared from rat chondrosarcoma as described previously [11, 12]. The collagen II was dissolved in 0.1 M acetic acid at a concentration of 1.5 mg/ml and emulsified 1:1 in Freund’s incomplete adjuvant (Difco, Detroit, MI, USA). Two hundred microlitres of emulsion was injected intradermally at the base of the tail, so that each rat was given 150 µg collagen II.

Arthritis was quantified by a clinical scoring system ranging from 0 to 16. Each paw was scored as follows: 0 = no arthritis; 1 = swelling in one type of joint; 2 = swelling in two types of joint; 3 = swelling in three types of joint; 4 = swelling of the entire paw. A total score for each rat was calculated by summarizing the scores for each of the four paws, giving a maximum score of 16 for each rat [13]. Body weight was also recorded.

Treatment protocols

At first, a dose-titration study was performed in order to determine the dose of corticosteroid that would have a clear effect on the development of clinical arthritis without inducing complete remission of the disease; such a dose was considered to provide the best model for most situations in RA patients treated with low to moderate doses of corticosteroids. Treatment was with three different doses of corticosteroids (CS) [betamethasone sodium phosphate (Betapred®; Swedish Orphan, Stockholm, Sweden), 0.5, 0.1 and 0.02 mg/kg body weight] or, as placebo, saline (NaCl), given daily intraperitoneally. Five rats were used initially in every treatment group. Treatment was initiated when the arthritis score was ≥ 2 and the disease incidence in this part of the study was between 60 and 80%; thus, three or four rats in each group were included in the titration study.

For the main study, a dose of 0.1 mg/kg/day of betamethasone was chosen and compared with saline. Treatment in the main study was initiated when the arthritis score was ≥ 2.

Immunoaassays

Blood samples were taken by retro-orbital puncture before immunization and at selected time-points after immunization. Serum levels of COMP were determined by an enzyme-linked immunosorbent assay (ELISA) using a modified version of the assay used for quantification of human COMP [14]. The assay was modified by using rat COMP to coat the microtitre plates and for the standard curve included in each plate, and by using a polyclonal antiserum raised against rat COMP for detection [15].

Plasma levels of fibrinogen were measured by nephelometry, as described previously [16]. Results of fibrinogen quantification are presented as percentages of the initial values in the experiments.

Histological examinations

At day 21 post-immunization (p.i.), eight animals from each group in the main study were killed for histological examination. At day 25 p.i. the remaining animals were subjected to the same procedure. For histological examination, the hind paws were dissected and fixed in 4% phosphate-buffered formaldehyde for 24 h. Subsequently, the specimens were decalcified in 14% ethylene dinitritolatetraacetic acid (EDTA) in 0.36 M NaOH for 3 weeks, dehydrated in ethanol and embedded in paraffin blocks. Sections (8 mm thick) were cut, mounted on slides and stained with haematoxylin and eosin [13]. The degree of cell infiltration in synovial fluid, synovitis and destruction of cartilage and bone were investigated using a Reichert–Jung Polyvar 2 light microscope.

Joints were graded using a modified version of a system adopted from Joosten et al. [17]. For each animal, both hind paws were examined. Cell infiltration in synovial fluid was graded from 0 to 3 as follows: 0 = no inflammatory cells in the joint cavity; 1 = a few inflammatory cells in the joint cavity; 2 = joint cavity partly filled with inflammatory cells; 3 = joint cavity totally filled with inflammatory cells. Synovitis was graded from 0 to 3 as follows: 0 = healthy, uninvaded appearance of the synovium; 1 = mild thickening of the synovium; 2 = substantial thickening of the synovium; 3 = severe thickening of the synovium. Destruction of cartilage was graded from 0 to 3 as follows: 0 = normal appearance; 1 = minor destruction of the cartilage surface; 2 = clear loss of cartilage; 3 = cartilage almost absent in the whole joint. The juxta-articular bone involvement was graded from 0 to 3 as follows: 0 = normal appearance; 1 = minor signs of destruction; 2 = up to 30% destruction; 3 = more than 30% destruction.

Two observers performed all histological evaluations without knowledge of sample origin, and if the results were inconsistent the slide was reinvestigated and the grading was discussed and adjusted.

Statistical calculations

The Mann–Whitney U test was used for comparisons between the groups. The Wilcoxon matched pair test was used for comparisons at different time-points within a group. Correlations were calculated using the Spearman rank order correlation coefficient. A P value < 0.05 was considered significant. Only animals developing disease after immunization were included in the calculations.

Results

Development of disease

In the dose titration study, three different corticosteroid doses and placebo were compared. Treatment was started when the arthritis score exceeded 1 (Fig. 1A). Rats in the placebo-treated group reached a maximum arthritis score of median 6 at the end of the study (day 22 p.i.). Treatment with the highest dose of corticosteroid (0.5 mg/kg/day) completely resolved all clinical signs of arthritis at day 20 p.i. Treatment with 0.1 mg/kg/day halted the progression of arthritis and at day 22 p.i. a median score of 1 was observed, compared with 6 in the placebo group. A corticosteroid dose of 0.02 mg/kg/day also suppressed disease, yielding a median score of 3 at its maximum, at day 22 p.i. The weight curves seen in rats subjected to the three different corticosteroid doses were very similar. A corticosteroid dose of 0.1 mg/kg/day was chosen for the following study.

In the next experiment, 40 rats were immunized with collagen II (20 rats in each group). In the group intended for corticosteroid treatment, 17/20 (85%) exhibited an arthritis score ≥ 2 and were therefore treated and included in the analysis (Fig. 1B). In the group intended for placebo treatment, 18/20 (90%) exhibited a clinical score ≥ 2 and were included in treatment and analysis. The onset of arthritis occurred between days 15 and 18 p.i. The maximum arthritis score was detected at day 25 p.i. in the placebo group. Already at day 17 the clinical scores differed significantly between the corticosteroid- and placebo-treated groups. The difference remained throughout the experiment. At day 21 p.i., eight animals in each group were bled and their paws were subjected to histological examination. At day 25 p.i., all the remaining animals were subjected to the same procedure (see below).

Weight

Both corticosteroid-treated and placebo-treated rats lost weight during the experiment but there was no statistically significant difference between the groups (data not shown).
FIG. 1. (A) Arthritis scores for the titration study. The maximum score in this arthritis model was 16. In order to define a dose that would suppress but not completely abolish arthritis, three doses of betamethasone were evaluated in parallel with a placebo treatment (saline). Treatments were initiated after onset of arthritis. The data are presented as individual scores for all animals. (B) Arthritis scores for corticosteroid- and placebo-treated animals. Eight animals in each group were killed for histology at day 21. For the corticosteroid group, n = 17, and n = 9 after day 21. For the placebo group, n = 18, and n = 10 at day 25. (C) Serum levels of fibrinogen as a measure of general inflammation. The corticosteroid dose was 0.1 mg/kg body weight. For the corticosteroid-treated group, n = 17, and n = 9 at day 25. For the placebo group, n = 18, and n = 10 at day 25. (D) Serum levels of COMP as a measure of cartilage involvement. The corticosteroid dose was 0.1 mg/kg body weight. For the corticosteroid-treated group, n = 17, and n = 9 at day 25. For the placebo group, n = 18, and n = 10 at day 25. All values in B–D are median ± interquartile range.
Serum concentrations of fibrinogen and COMP

In the placebo-treated group, serum concentrations of fibrinogen were increased at day 15 p.i. and peaked at day 21 p.i. (\(P < 0.05\) vs baseline at the respective time-points), whereas in the corticosteroid-treated group the fibrinogen levels steadily increased and reached a maximum at the end of the study (Fig. 1C). The fibrinogen levels were significantly higher in the placebo group than in the corticosteroid-treated group at days 21 and 25 p.i. (\(P < 0.05\)). In both groups, serum COMP increased from immunization until day 15 p.i. (\(P < 0.05\)) (Fig. 1D). In the placebo-treated group, serum levels of COMP increased continuously during the treatment period (\(P < 0.01\)). In the corticosteroid-treated group, serum COMP did not change during the treatment period. COMP levels were significantly lower in the corticosteroid-treated group than in the placebo-treated groups at day 21 p.i. and day 25 p.i. (\(P < 0.01\)).

Histological examination

At day 21 p.i., there was less synovial inflammation, less cartilage destruction and fewer bone erosions in the corticosteroid-treated group than in the placebo-treated group. The degree of synovial fluid inflammation varied greatly within the corticosteroid-treated group and no significant difference from the placebo-treated group was detected. Similar observations were made in paws obtained at day 25 p.i. The degree of inflammation, as recorded by clinical scoring, correlated well with the histological evaluations (Table 1). There was a significant correlation between COMP levels and the
Discussion

The main finding in the present study is that moderate doses of corticosteroids can retard bone and cartilage destruction in rats with CIA. The second important finding is that the measurement of serum COMP appears to provide a good and dynamic picture of how corticosteroids affect the destructive process of inflammatory polyarthritis.

The choice of the CIA model for the study of the effects of corticosteroids on joint destruction was made largely on the assumption that similar molecular events may contribute to joint destruction in RA and in CIA [12]. This assumption is supported both by the demonstration of similar cellular and molecular patterns in inflamed joints of humans with RA and rodents with CIA respectively, and by evidence that drug testing for the reduction of inflammation and destruction in the CIA model has often been able to predict the effects of these drugs in human RA [18].

In this study, betamethasone was the corticosteroid used. Betamethasone has predominantly a glucocorticoid effect, whereas prednisolone has more of a mineral-corticosteroid effect. This is one of the reasons why betamethasone was used, as we wanted to exclude the effects of the corticosteroid on minerals. To define the dose to be used in the study, we made a titration study and chose a dose of 0.1 mg/kg/body weight. This dose had a clear effect on the arthritis but did not completely abolish the disease, a situation that is often encountered in the treatment of patients with inflammatory joint disease. The bioavailability of corticosteroids differs between rats and humans, so the dose of 0.1 mg/kg cannot easily be translated to its human equivalent, but we believe that the similarity in clinical effectiveness on inflammation between humans with RA and rats with CIA may nevertheless ensure that the doses used in the rat experiments are also relevant for the human situation.

The fibrinogen analysis in this paper demonstrates that fibrinogen levels increase with disease activity in the early stages of disease. In the chronic phase of the corticosteroid-treated group, an increase in fibrinogen levels indicates increased inflammatory activity. However, this was not reflected in the arthritis score at this time-point. Serum levels of COMP, determined with the methods
used in the present paper, have been shown to correlate well with ongoing cartilage destruction, both in the CIA model in rats and in other arthritis models in rats and mice [19, 20]. We concluded from these observations that the quantification of serum COMP, in parallel with careful histological evaluation of cartilage destruction, may provide us with increased knowledge of whether corticosteroids can affect cartilage destruction, and whether serum COMP provides a useful tool for the evaluation of such a drug effect.

The capacity of certain doses of corticosteroids to diminish cartilage destruction in CIA has been demonstrated previously by Bendele et al. [21]. These authors, however, did not describe the effects of corticosteroids on joint morphology over time, and it also appears that the doses of corticosteroids they used gave rise to severe weight loss. The present study thus confirms and extends the findings of Bendele et al. concerning the effects of corticosteroids on experimental arthritis.

A first attempt to use serum COMP to determine the cartilage-protective effects of anti-rheumatic therapy was made in experimental arthritis in mice subjected to blockade of interleukin 1 (IL-1) and tumour necrosis factor (TNF) [6]. This study demonstrated the usefulness of serum COMP in measuring effects on cartilage protection. The study also showed that IL-1 blockade, but not TNF blockade, prevented cartilage and bone destruction. Because studies in RA have shown that TNF blockade halts the progression of joint damage [22], it also emphasizes the difficulty of transferring the results of a given targeted therapy from rodents to man. However, serum COMP has been shown to decrease in RA patients treated with infliximab or etanercept [7].

The results of the present study, i.e. of a cartilage-protective effect of corticosteroids in CIA in rats, and the fact that this protective effect was immediately mirrored by a decrease in serum COMP values, suggests that serum COMP may be used to evaluate the effects of corticosteroid on cartilage destruction in human arthritis also. If so, it may be possible not only to evaluate the effects of constant doses of corticosteroids given for longer time periods in clinical trials, but also to estimate the cartilage-protective effects, if present, of corticosteroids given at different periods in clinical trials, but also to estimate the cartilage-protective effects, if present, of corticosteroids given at different periods in clinical trials, and long-term observational studies that disease-modifying anti-rheumatic drugs slow radiographic progression in rheumatoid arthritis: using a 1983 review. Rheumatology 2002;41:1346–56.


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References
