USE OF CARTILAGE OLIGOMERIC MATRIX PROTEIN FOR THE TREATMENT OF RHEUMATOID ARTHRITIS

Background

The present invention relates to use of cartilage oligomeric matrix protein or fragments or analogues thereof, for the manufacture of a pharmaceutical composition for the prevention or treatment of arthritic conditions in mammals.

Arthritis is a common name for several inflammatory joint diseases, e.g. rheumatoid arthritis, bacterial arthritis, reactive arthritis, and crystal-induced arthritis. Rheumatoid arthritis (RA) comprises a large group of non-bacterial conditions. Classical rheumatoid arthritis normally affects several joints (polyarthritis), but may also be restricted to a single joint (monoarthritis). The attack of the joint cartilage is only one of several factors which degenerate the cartilage and the bone and destroy the joint function. Also the joint capsule, the ligaments, and the bone tissue are affected.

Description of the Prior Art

At present, the symptoms of e.g. rheumatoid arthritis for some patients can be alleviated and the joint function be maintained or improved by medical treatment, e.g. with corticosteroids or methotrexat, physiotherapy, or surgical treatment. However, several patients are difficult or impossible to improve by treatment. Further, there exists no method for prevention of rheumatoid arthritis.

Thus, there is a need to prevent the symptoms of rheumatoid arthritis. There is also a need of further alleviating and reducing the symptoms of rheumatoid arthritis.

Summary of the Invention

An object of the present invention is to prevent or alleviate the symptoms of arthritic conditions. This object is achieved by use of cartilage oligomer matrix protein (COMP), or fragments or analogues thereof, for the manufacture of a pharmaceutical composition for the

Thus, COMP is a known compound and has previously been described as being useful in diagnostic methods for estimating the degree of cartilage degradation in arthritic patients (9).

It is already known that autoimmune reactions to certain cartilage antigens present in the joint cartilage cab be used to induce rheumatoid arthritis in animal models. Collagen of different types (II, IX, and XI) and aggrecan have been classified as cartilage autoantigens inducing autoimmunity and rheumatoid arthritis in animal models. These antigens have also been used in efforts to treat rheumatoid arthritis.

However, it has not previously been shown that COMP is arthritogenic and therefore potentially of use in the treatment of rheumatoid arthritis. The inventors of the present invention have reached these results after experiments in which COMP has been added to rats. These experiments are described below.

The present invention therefore relates to the use of cartilage oligomeric matrix protein (COMP), or fragments or analogues thereof, for the manufacture of a pharmaceutical composition for prevention or treatment of arthritic conditions in a mammal, wherein the pharmaceutical composition is administered in an amount effective to prevent or treat the arthritic condition.

In one embodiment, the pharmaceutical composition comprises an RNA or DNA sequence capable of expressing the COMP or fragments or analogues thereof, or a vector cell containing said RNA or DNA sequences.

The pharmaceutical compositions of the present invention optionally also comprise one or more substances selected from the group consisting of collagene II, collagene IX, collagene XI, and aggrecan, or fragments or analogues thereof.

The COMP of use in the present invention may be of human, bovine, or rat origin, produced by microorganisms or chemically synthesized.

(CII), prepared from a chondrosarcoma (4, 5), was dissolved in 0,1 M acetic acid at a concentration of 1.5 mg/ml. This solution was emulsified with an equal volume of incomplete Freund's adjuvant and 200 µl of the emulsion was injected intradermally at the base of the tail (150 µg/rat).

Evaluation of arthritis

Arthritis was assessed using a scale from 0-16, each of four paws being scored from 0-4 in which 0=no arthritis, 1-3=a summary of points as follows: swelling of the ankle-1 point, swelling of one or more intratarsal and/or metatarsal joints-1 point, and swelling of one or more interphalangeal joints-1 point, 4=swelling of all joints, i.e. the entire paw. The rats were examined every second to fourth day from day 10 until day38 after immunization.

Preparation of draining lymph node cells

Inguinal lymph node cells from rats immunized with COMP and from non-immunized control rats were obtained by dissection of the lymph nodes and gentle grinding through a tea-strainer. The cell suspensions were washed three times in PBS pH 7.4.

Measurement of cellular reactivity to bovine COMP: Proliferation

The inguinal lymph node cells were suspended at $1x10^6$ cells/ml in DMEM (X) supplemented with 5% FCS (Hyclone, Logan, UK), penicillin (100 u/ml), glutamine (2 nM) and streptomycin (100 µg/ml), all from Sigma, St Louis, MO. Cells from individual animals were plated in 96-well round-bottom cell culture plates (Nunc, Roskilde, Denmark), 0.2 ml per well. Antigens were added to triplicates of wells for each animal, dissolved in 10 µl PBS pH 7.4, to give the final concentrations: COMP, 10 µg/ml and 50 µg/ml, or ConA 5 µg/ml. As control, 10 µl of PBS were added to the wells. The cells were incubated for 96 hours in 37°C and 5% CO_2, and cell proliferation assessed by labelling proliferating cells in each well with 1 µCi of ^{3}H-thymidine for the final 6 hours before cell

serum from LEW.1AV1 rats with given arbitrary values of 100 AU/ml respectively. Each plate contained positive reference serum, serum samples to be measured and negative serum controls in duplicates. Negative serum controls were individual sera from non-immunized LEW.1AV1 rats.

RESULTS

Arthritogenicity of bovine COMP in LEW.1AV1 and F344 rats

In the first experiment, six LEW.1AV1 rats were immunized with 50 µg COMP/rat (table 1). 15-19 days later, three of these (3/6) developed arthritis which mainly affected the hind ankles. The disease was self-limiting, there were no macroscopic signs of arthritis or ankylosis 38 days after immunization. In the second experiment, nine LEW.1AV1 rats and four age-matched F344 rats were immunized with 150 µg COMP/rat (Table 1). This higher dose of COMP resulted in a similar incidence of arthritis in the LEW.1AV1 rats (5/11) but the disease appeared earlier, 12-14 days after immunization. Furthermore, the arthritis was more servere and typically affected ankle joints and metatarsal joints in the hind paws, arthritis in finger joints and fore paws was also observed. Two rats were monitored clinically until 38 days after immunization at which time they had signs of ankylosis. In contrast to rats of the LEW.1AV1 strain, F344 rats appeared less susceptible to COMP-induced arthritis since only minimal signs of disease could be detected, at a late time-point 25-29 days after immunization, in one out of four rats.

Humoral reactivity to bovine COMP in individual LEW.1AV1 and F344 rats, 16 days after immunization with bovine COMP

Analysis of individual sera demonstrated humoral reactivity to COMP in both F344 rats and LEW.1AV1 rats (Table 1). There was no correlation between presence of antibodies and arthritis.

arthritis, F344 rats appeared resistant. This indicates that susceptibility is genetically determined.

COMP is of interest as an arthritis-inducing antigen for several reasons. Firstly, COMP is present in the most superficial parts of the articular cartilage where it may be accessible to immune cells in the joints. Secondly, COMP is released into synovial fluid and serum of patients with early destructive arthritis (7), implying that this protein is also accessible to circulating immune cells. It has not earlier been reported whether an induced autoimmunity against COMP can cause arthritis such as in this present study, nor whether autoimmune reactions towards COMP occur in patients with arthritis.

Bovine and rat COMP used in these experiments were purified from cartilage such that no contamination of other proteins could be detected, as observed by the absence of other proteins than COMP on SDS-PAGE gels used for the biochemical characterization (data not shown). However, the existence of minor contaminants in the preparation may not be excluded, but such contaminants are unlikely to be responsible for arthritis induction since their concentrations would be orders of magnitude lower than that of other arthritogens used in rats. That contaminating collagen type II is not involved in arthritis development is also indicated by the lack of a humoral immune response to rat CII in rats immunized with the COMP preparation.

Table 2. Humoral reactivity to COMP and collagen type II (CII) in LEW.1AV1 rats immunized with incomplete Freund's adjuvant (IFA) alone, or together with COMP or CII.

Rats immunized with:	Maximal severity	α-CII (AU)	α-COMP (AU)
IFA (6)	0	0	0
CII+IFA (5)	7-12	61-223	0-2
COMP+IFA (5)	1-7	0	215-483

Sera were obtained from rats which developed bovine COMP-induced arthritis (38 days post immunization, dpi), rat collagen-induced arthritis (28 dpi) or, as a control, from rats immunized with IFA (28 dpi).

The expression "fragments" of COMP used throughout the application include any partial amino acid sequences or modified partial sequences thereof, i.e. those being shorter in length than COMP and/or containing any deletions/substitutions or modifications to the constituent amino acids, that are capable of inducing a biological response similar to COMP, i.e. the ability to suppress or eliminate T-cell or B-cell mediated and/or T-cell or B-cell dependent autoimmune response, or act in a manner so as to inhibit the arthritogenic effects of COMP. The expression "analogues" of COMP used throughout the application includes compounds (both peptides and non-peptide chemical compounds) that possess similar biological activity to COMP, i.e. the ability to eliminate or suppress the T-cell or B-cell mediated and/or T-cell or B-cell dependent autoimmune response, or act in a manner so as to inhibit the arthritogenic effects of COMP. Thus, the latter expression includes amino acid sequences which differ from the amino acid sequence of COMP by one or more amino acids, while retaining substantially equivalent biological activity, as well as chemical compounds which mimic the biological activity of COMP in its ability to suppress or alleviate the symptoms of rheumatoid arthritis.

The pharmaceutical composition comprising COMP or the fragments or analogues thereof may be administrated to a mammal, preferably a human, in a number of known routes including oral, mucosal, nasal, rectal or vaginal route, or by inhalation or injection.

For oral administration the pharmaceutical composition may be e.g. tablets, pills, capsules, syrups, powders, granules, or solutions.

For oral administration the active compound may be admixed with an adjuvant or a carrier, e.g. lactose, saccharose, sorbitol, mannitol, starches such as potato starch, corn starch or amylopectin, cellulose derivatives, a binder such as gelatine or polyvinylpyrrolidone,

In another embodiment the pharmaceutical composition according to the present invention comprises COMP or fragments or analogues thereof in combination with one or more arthritogenic substances chosen from the group consisting of collagene II, IX, XI, and aggrecan, or fragments or analogues thereof.

COMP, and fragments or analogs thereof, may be isolated from the naturally occuring material as described in (1) or may be expressed from the nucleotide sequence given for COMP in (2). The skilled man will be aware as to how such expression may be obtained and how such fragments or analogs may be prepared from the nucleotide sequence. COMP and its fragments and analogs may also be chemically synthesized using the deduced amino acid sequence given in (2).

The present invention therefore also relates to the use of COMP as described above, wherein the pharmaceutical composition comprises RNA or DNA sequences expressing COMP or fragments or analogues thereof, or a vector cell containing said RNA or DNA sequences. Thus, the RNA or DNA sequences are administrated to the mammal either directly or via the vector cell.

COMP or the fragments or analogues thereof may have any origin, but has preferably human, rat, or bovine origin. It may be prepared by purification from any of these origins by means of standard methods. It can also be expressed by a microorganism or be chemically synthesized, as described above.

According to the present invention COMP or fragments or analogues thereof may be added in a pharmaceutical composition administration to mammals in order to prevent or alleviate the symptoms of arthritic conditions, preferably rheumatoid arthritis. Thus, when the pharmaceutical composition according to the present invention is administrated to a mammal, preferably a human, having rheumatoid arthritis, COMP or the fragments

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CLAIMS

1. Use of cartilage oligomeric matrix protein (COMP), or fragments or analogues thereof, for the manufacture of a pharmaceutical composition for the prevention or treatment of arthritic conditions in a mammal, wherein the pharmaceutical composition is administrated in an amount effective to prevent or treat the arthritic condition.

2. Use according to claim 1, wherein the pharmaceutical composition comprises an RNA or DNA sequence capable of expressing COMP or fragments or analogues thereof, or a vector cell containing said RNA or DNA sequences.

3. Use according to claim 1 or 2, wherein in the pharmaceutical composition optionally also comprises one or more substances selected from the group consisting of collagene II, collagene IX, collagene XI, and aggrecan, or fragments or analogues thereof.

4. Use according to claim 1, wherein the COMP has human, bovine, or rat origin, or has been produced by microorganisms or has been chemically synthesized.

5. Use according to any one of the preceding claims, wherein the pharmaceutical composition is administrated by the oral, mucosal, nasal, rectal or vaginal route, or by inhalation or injection.

6. Use according to claim 1, wherein the mammal is a human.

7. Use according to claim 1, wherein the arthritic condition is rheumatoid arthritis.