Induction of osteoarthritis in the rat by surgical tear of the meniscus: Inhibition of joint damage by a matrix metalloproteinase inhibitor

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Summary

Objective: Characterize a model of osteoarthritis (OA) induced by a surgically transecting the medial collateral ligament and meniscus. Evaluate the effectiveness of a matrix metalloproteinase (MMP) inhibitor in this model.

Methods: The medial collateral ligament of the right knee of rats was transected and a single full thickness cut was made through meniscus. Rats were sacrificed at various times after the surgery to assess the severity of gross cartilage damage using an image analyser and microscopically by histology. The effect of an MMP inhibitor in this model was assessed by administering compound twice daily for the 21 days and evaluating gross and histological joint damage at day 21. The in vitro potency of the MMP inhibitor (MMPI) against a panel of human recombinant MMPs was assessed kinetically using a quenched fluorescent substrate.

Results: Surgical transection of the medial collateral ligament and meniscus resulted in a time dependent increase in the severity of the cartilage lesion (depth) as measured histologically but only a slight increase in the area of the lesion as assessed grossly by image analysis. Administration of a MMPI orally twice daily (b.i.d.) at 25 mg/kg to rats in the meniscal tear model resulted in significant inhibition of cartilage degradation and osteophyte formation (total joint score) of 39±7% (mean±S.E.M., from four separate experiments).

Conclusion: These results demonstrate that MMP inhibition is effective in reducing the joint damage that occurs in the meniscal tear model of OA and support a potential therapeutic role for MMP inhibition in the treatment of human OA. © 2002 OsteoArthritis Research Society International. Published by Elsevier Science Ltd. All rights reserved.

Key words: Matrix metalloproteinase inhibitor, Meniscal tear model of OA, Rats.

Introduction

Osteoarthritis (OA) is a degenerative joint disease that affects a large and growing population. Characteristic features of OA include cartilage degradation, sclerosis of subchondral bone and osteophyte formation. Although the etiology of OA is poorly understood numerous animal models that mimic aspects of the disease have been developed to study the pathophysiology of the disease and to evaluate potential therapeutics. A variety of surgical models of OA in animals have been devised to try to accelerate the degenerative changes that occur over long periods of time in spontaneous OA. Some of the more commonly used models in larger animals are the Pond-Nuki model (anterior cruciate ligament transection) in the dog, lateral menisectomy in the sheep and partial menisectomy in rabbits. Small animal models of surgical OA have also been described including partial menisectomy in guinea-pigs and anterior cruciate ligament transection in rats.

The progressive cartilage destruction that occurs in OA is thought to result from the proteolytic activity of matrix degrading enzymes such as the matrix metalloproteinases (MMPs). MMPs have been found to be elevated in animal models of OA, in human OA cartilage and in the synovial fluid from OA patients. There have been few studies examining the affect of MMP inhibitors in animal models of OA. Doxycycline, reported to have MMP modulatory effects was found to reduce the severity of cartilage lesions in dogs with OA induced by dorsal root ganglioneuromy followed by cruciate ligament transection and a modified tetracycline (CMT-7) with similar MMP modulatory effects was found to be efficacious in spontaneous OA in guinea-pigs. The collagenase selective MMP inhibitor, Ro 32-3555 was shown to inhibit cartilage and bone changes that occur spontaneously in the STR/ORT mouse and we have shown a number of MMP inhibitors to significantly inhibit the joint damage that occurs after the injection of iodoacetate in the knees of rats. The use of small animal surgical models of OA have advantages over models in large animals or spontaneous models, as less compound needs to be synthesized and results may be obtained in a shorter time frame. However, small animal surgical models such as the partial menisectomy model in guinea-pigs tend to result in severe cartilage damage within 1 week and may be too aggressive for effective therapy. In the present study we demonstrate that transection of the medial collateral ligament and meniscus results in a time dependent increase in cartilage damage with osteophyte generation and that this joint damage can be effectively treated with MMP inhibitors. These data support a potential role for MMPs in the mediation of joint damage in the rat meniscal tear model.
and supports the further evaluation of the therapeutic potential of MMP inhibition in human OA.

Materials and methods

ANIMALS

Male Lewis rats weighing approximately 350 g (Harlan, Indianapolis, IN) were housed singly in wire cages in sanitary ventilated animal rooms with controlled temperature, humidity and regular light cycles. Rodent chow (Ralston-Purina, Richmond, IN) and water were allowed ad libitum. Animals were acclimated for one week before use.

It is the policy of Procter & Gamble Pharmaceuticals that all animals be housed, fed, and handled in compliance with the standards set forth by the Animal Welfare Act as amended. Where standards are not indicated in the Animal Welfare Act the recommendations on HHS Publications (NIH) No. 85-23, ‘Guide for the Care and Use of Laboratory Animals’ were followed. The personnel at Procter & Gamble Pharmaceuticals and BolderPath, Inc., adhered to and were in compliance with the above stated policy.

SURGICALLY INDUCED MENISCAL TEAR OA IN RATS

Rats were anesthetized with isoflurane and the right knee was shaved and scrubbed in preparation for surgery. The medial collateral ligament was transected and the medial meniscus was grasped with a hemostat and reflected proximally toward the femur. The meniscus was transected with a scalpel or small surgical scissors. The skin was closed with 4-0 silk sutures. Groups of animals were sacrificed 1, 2, 3 or 6 weeks later in time course studies and 3 weeks after surgery in studies measuring the efficacy of an MMPI.

The surgical right knee was removed at sacrifice, disarticulated, and the tibia stained using a solution of 0.125% Evan’s Blue stain in saline. The knee joint was then fixed in 10% buffered formalin for 48 h prior to image analysis. An image of the tibial plateau of each knee was captured using an Optimas image analysis system (Optimas Media Cybernetics LP, Silver Springs, MD). The tibial plateau was used for image analysis because it provided a relatively flat surface compared to the femoral condyles allowing the image analysis camera to focus on the entire cartilage surface. The area of the tibial plateau that was damaged was quantitated using software of the image analysis system. The damaged area of the tibial plateau (stained with Evan’s blue) was measured and this value was divided by the total area of the tibial plateau to yield the percent area of cartilage damage.

Studies on the affect of the MMPI on cartilage degeneration in the rat meniscal tear model were performed by administering the compound orally. Animals were dosed orally by gavage with either the MMP inhibitor or vehicle (10% cyclodextrin) twice daily at 12 h intervals for 21 days. The compound used in these studies is a sulfone-based MMP inhibitor with hydroxamic acid as the chelating moiety for the active site Zn⁺⁺ and is similar to compounds in a series of carboxylic acids that we have described previously.

HISTOPATHOLOGY

The fixed knee joints were decalcified in Surgipath decalcifier I (Grayslake, IL) for approximately 1 week.

When decalcification was complete the knees were transected in the frontal plane so the medial and lateral orientation was maintained and both halves were embedded in paraffin. An initial section was cut followed by two step cuts at 150 μm intervals. The sections were stained with toluidine blue and evaluated for cartilage damage and osteophyte formation. The cartilage damage was evaluated using the following system:

1 = minimal superficial zone only
2 = mild extends into the upper middle zone
3 = moderate well into the middle zone
4 = marked into the deep zone but not to tidemark
5 = severe full thickness degeneration to tidemark

The amount of cartilage damage was assessed as 1/3, 2/3 or 3/3 of the surface of the histological section and the above score was multiplied by 1, 2 or 3, respectively to reflect the extent of the tibial plateau that was involved. Osteophytes were scored 1, 2 or 3 for mild (≤40 μm) moderate (40–160 μm) or severe (>160 μM) depending on the size using an ocular micrometer.
Data were analysed using a nonparametric procedure (Wilcoxon rank sum). The data are expressed as the mean±S.E.M. and statistical differences from the vehicle treated control (P<0.05) are denoted with an asterisk.

MMP INHIBITION ASSAY

Previously we have described the inhibition of MMP activity in detail as well as the preparation of the human recombinant enzymes used in these studies. The MMP inhibitor used in this study was evaluated for its ability to inhibit human MMPs using the quenched fluorescence assay modified to fit a 96-well format. Briefly, MMPs 1, 2, 3, 7, 8, 9 and 13 were used at final concentrations of 8 nM, 1 nM, 16 nM, 2 nM, 4 nM, 0.75 nM and 0.5 nM, respectively. The MMP assays were performed using the fluorogenic substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH₂ at a concentration of 4 μM at 25°C. The assay buffer was 50 mM Tris, pH 7.5, 0.2 M NaCl, 5 mM CaCl₂ and 0.02% Brij-35. The increase in fluorescence due to cleavage of the

STATISICAL ANALYSIS

Fig. 3. Microscopic features of osteoarthritic lesions of the tibial plateau in the rat meniscal tear model. Coronal section of the medial tibial plateau stained with Toluidine blue, 50×: (a) 6 weeks after transection of the medial collateral ligament but without a medial meniscal tear, showing only minimal peripheral proteoglycan loss and fibrillation (red arrowhead); (b) 1 week after meniscal tear there is focal cartilage degeneration (black arrow) extending over half of the load bearing surface. Early proliferation (chondrophyte) is evident in the marginal zone (red arrow); (c) 2 weeks after meniscal tear cartilage degeneration extends over two-thirds of the surface and is focally severe in the outer one-third (black arrow). A small osteophyte is present (red arrowhead); (d) 3 weeks after meniscal tear there is focally severe cartilage degeneration in the outer one-third of the tibial plateau (black arrow) with marked cartilage degradation in the middle one-third and a definite osteophyte (red arrowhead) and periosteal proliferation (green arrowhead); 6 weeks after meniscal tear severe cartilage degeneration is observed in the outer half of the tibial plateau (black arrows) a mature osteophyte (red arrowhead) and periosteal proliferation are observed.
substrate (Gly-Leu bond) was monitored kinetically for 30 min with a BMG Fluostar fluorescence plate reader ($\lambda_{em}$ 328 nm, $\lambda_{ex}$ 393 nm). Each 96-well microtiter plate contained 100 µl of substrate and 50 µl of enzyme in each well. 50 µl of MMP inhibitor was added to each well (except for positive control) to give a final volume of 200 µl/well. The MMP inhibitor was tested at eight different concentrations and an IC$_{50}$ was calculated using the formula:

$$V/V_o = 1/1 + [I]/IC_{50}$$

where $V_i$ is the initial velocity of substrate cleavage in the presence of inhibitor at concentration [I] and $V_o$ is the initial velocity in the absence of inhibitor.

**Results**

**KINETICS OF JOINT LESION DEVELOPMENT FOLLOWING MENISCAL TEAR IN RATS**

Surgical meniscal tear was performed on the right knees of rats and groups of animals were sacrificed 1, 2, 3 or 6 weeks later. Cartilage lesions were readily apparent by gross observation at 1 week after surgery and appeared as a crescent-shaped area of damage stained by Evan’s blue. Osteophytes also occurred on the peripheral aspects of the femur but are generally smaller than on the tibia. In general, the degree of cartilage degeneration of the femoral groove and condyles does occur. In general, the development of cartilage degeneration as this relatively flat surface is more amenable to image analysis studies, cartilage degeneration of the femoral groove and condyles does occur. In general, the severity of cartilage degeneration of the femur is less marked than that of the tibial plateau. Osteophytes also occur on the peripheral aspects of the femur but are generally smaller than on the tibia.

**IN VITRO INHIBITORY PROFILE OF THE MMP**

The MMP inhibitory profile of the MMPI tested in the rat meniscal tear model is shown in Table I. The compound was evaluated for its ability to inhibit human recombinant MMPs 1, 2, 3, 7, 8, 9 and 13 in kinetic assays. The MMPI tested in the rat meniscal tear model was a very potent inhibitor of MMPs 2, 3, 8, 9 and 13, whereas it was considerably less potent for MMPs 1 and 7. Since rats lack MMP-1, any affect due to collagenase inhibition would be due primarily to MMP-13 inhibition.

**INHIBITION OF JOINT DAMAGE IN THE MENISCAL TEAR MODEL BY AN MMP INHIBITOR**

Rats that had a surgically induced meniscal tear were treated twice daily with an MMPI by oral gavage at a dose of 25 mg/kg. The animals were sacrificed 3 weeks after surgery and joint damage was assessed histologically. The...

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**Table I**

<p>| MMP inhibition profile for the MMPI evaluated in the rat meniscal tear model |
|---------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|</p>
<table>
<thead>
<tr>
<th>IC$_{50}$ (nM)</th>
<th>MMP-1</th>
<th>MMP-2</th>
<th>MMP-3</th>
<th>MMP-7</th>
<th>MMP-8</th>
<th>MMP-9</th>
<th>MMP-13</th>
</tr>
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<tr>
<td>145±0.5</td>
<td>&lt;0.4</td>
<td>8.4±2.9</td>
<td>1322±165</td>
<td>&lt;0.4</td>
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Data are the mean±s.d. from three experiments.
3 week time point was chosen because it provided sufficient time for joint damage to develop but was brief enough to allow evaluation of the efficacy of compounds in a relatively short period of time thereby reducing the required quantity of inhibitor. The MMPI significantly (P<0.05) inhibited joint damage in four of four experiments as assessed by total histological score (Table II). Significant inhibition of the tibial plateau, femoral surface and osteophyte scores was observed in three of four studies (Table II). The mean % inhibition of joint damage by MMPI for all four experiments were 39±7, 38±8, 55±15 and 34±7%, (mean±S.E.), respectively for the total, tibia, femur and osteophytes scores.

Photomicrographs from representative animals treated with either vehicle or MMPI are shown in Fig. 5. There was moderate to severe degeneration of the cartilage of the tibial plateau in the vehicle treated rat (Fig. 5, left panel) whereas, the degenerative changes were less severe in the MMPI treated animal (Fig. 5, right panel).

The body weights of the animals in each of the four studies above were recorded at day 0, 1, 2, and 3 weeks into the experiments to determine if the compound effected weight gain which could affect the severity of the cartilage damage. Administration of MMPI orally twice daily for 3 weeks did not effect weight gain with a less than 2% difference in the mean group weights in all experiments and time points with the exception of experiment 4 at 3 weeks where the MMPI treated group was 3% heavier than the vehicle treated controls (data not shown).

Discussion

The matrix metalloproteinases have been implicated in the cartilage degradation that occurs in human OA11–14,23,24 and in animal models of this disease25,26. Injury to the meniscus and/ or ligaments in humans often leads to the development of OA27 with an increase in cartilage matrix molecules and MMPs released into the synovial fluid14. In the present study we have described a model of OA in rats induced by transection of the meniscus and a role for MMPs in the cartilage damage and osteophyte formation that occurs in this model is suggested by the effectiveness of the MMP inhibitor.

The initial assessment of joint damage in the meniscal tear model involved the measurement of the area of the gross cartilage lesions. Approximately 20% of the area of the medial tibial plateau of the surgical knee showed visible damage one week after surgery but the area of this lesion showed little progression by 6 weeks (Fig. 2). In contrast, an evaluation of the cartilage lesions by histology demonstrated a progression in the severity of the lesion suggesting that the depth of the cartilage damage increased with time (Fig. 3). Therefore, although we would...
have preferred to use a quantitative and relatively fast method of gross damage evaluation using image analysis, histological assessment provided a more accurate method to demonstrate the effectiveness of a MMPI.

Rapid and severe cartilage degeneration occurs in rats after meniscal tear. The cartilage lesions that occur in the rat meniscal tear model are morphologically similar to those that occur in human OA but occur much more rapidly. This may be due to the rats normally using their unstable joint immediately after surgery whereas humans and other animals such as the dog do not immediately load their knee joint after surgery. The rapid progression of the cartilage degeneration in this model provides a high hurdle for testing the efficacy of a therapeutic.

Recently, the potential role of MMPs in cartilage degeneration in another rat model of cartilage degeneration the anterior cruciate ligament transection model has been suggested by the presence of the collagenase cleavage site as detected with the Col2-3/4Cshort antibody. In these studies, the collagenase cleavage was detected associated with the cartilage lesions that occur in the first 2 weeks after an anterior cruciate ligament transection but the collagenase cleavage epitope was less prominent at later times. However, the collagenase generated neoepitopes can be lost if a single amino acid is cleaved and a number of MMPs including gelatinases can result in the loss of the staining with Col2-3/4Cshort. Therefore, it is possible that MMPs are important mediators of cartilage degeneration in the rat ACL model of OA.

The loss of aggrecan from cartilage is believed to be due to the proteolytic cleavage within the interglobulin domain (IGD) between the G1 and G2 domains at residues Asn341-Phe342 and Glu373-Ala374. A number of MMPs have been shown to cleave aggrecan at this site. Cleavage at the Glu373-Ala374 site is due to ‘aggrecanase’ activity and two forms of this enzyme recently have been cloned. Recently, both MMP and aggrecanase generated neoepitopes have been found in the cartilage matrix adjacent to OA lesions in the STR/ORI mouse model of spontaneous OA. Therefore, both MMP and aggrecanase proteolytic activity may contribute to the cartilage damage that occurred in the rat meniscal tear model. The MMP inhibitor used in this study did not inhibit IL-1 stimulated glycosaminoglycan loss from bovine nasal cartilage explants, a measure of aggrecanase activity at concentrations as high as 50 µM (Janusz et al., unpublished data) suggesting that inhibition of ADAMs such as aggrecanase is not responsible for the therapeutic effect observed with this MMPI.

In conclusion, this study shows that transection of the meniscus and medial collateral ligament in rats leads to osteoarthritic changes in the knee. Degradation of cartilage and osteophyte formation in the rat meniscal tear model was significantly inhibited by the oral administration of a MMP inhibitor. The inhibition of joint damage as assessed by total joint score observed in these studies ranged from 34–55% (Table II). These data are consistent with MMPs being important mediators of cartilage damage in the rat meniscal tear model but suggest that alternative enzymes such as aggrecanase, cathepsin B, cathepsin K or other mechanisms may also contribute. These data suggest a role for MMPs in the progressive joint degeneration that occurs in the rat meniscal tear model and support the further evaluation MMP inhibitors for the treatment of human OA.

References


