Osteoarthritis and Cartilage 18 (2010) 593–600

Osteoarthritis and Cartilage

Effects of a metalloproteinase inhibitor on osteochondral angiogenesis, chondropathy and pain behavior in a rat model of osteoarthritis

P.I. Mapp †*, D.A. Walsh †, J. Bowyer ‡, R.A. Maciewicz ‡

† Academic Rheumatology, University of Nottingham, Clinical Sciences Building, City Hospital, Nottingham NG5 1PB, UK
‡ Respiratory & Inflammation Research Area, AstraZeneca, Charnwood R&D, Bakewell Road, Loughborough, Leicestershire, LE11 5RH, UK

ARTICLE INFO

Article history:
Received 7 September 2009
Accepted 15 December 2009

Keywords:
Osteoarthritis
Rat model
Pain behavior
Chondropathy
Osteochondral angiogenesis

SUMMARY

Objective: To investigate the effects of a matrix metalloproteinase (MMP) inhibitor on joint pathology and pain behavior in the rat meniscal transection (MNX) model of osteoarthritis (OA) and evaluate which aspects of structural disease modification contribute to symptom improvement.
Methods: OA pathology was induced in male Lewis rats, by transecting the medial collateral ligament with (MNX) or without (SHAM) a full thickness cut through the meniscus. MNX animals were orally administered an equipotent MMP 2, 8, 9, 12, 13 inhibitor (0.25, 1 and 5 mg/kg/day) or vehicle from day 1. Chondropathy, osteophytosis, osteochondral vascularity were assessed from toluidine blue stained coronal sections of the total knee joint and weight-bearing asymmetry by incapacitance. Group differences were evaluated using 1-way analysis of variance (ANOVA) and associations as Spearman's correlation coefficients.
Results: Treatment with the MMP inhibitor reduced weight-bearing asymmetry from day 14 onwards, and attenuated chondropathy (both $P < 0.05$). Osteochondral vascularity was elevated in MNX compared with SHAM-operated animals ($P < 0.001$) and reduced, dose-dependently, by MMP inhibitor treatment ($r = -0.89$, $P < 0.05$). Reduced osteochondral vascularity and chondropathy were associated with the amelioration of weight-bearing asymmetry (both $P < 0.05$).
Conclusion: Here we show that treatment with a MMP inhibitor reduces joint damage, osteochondral angiogenesis and behavioral evidence of pain. The association between osteochondral angiogenesis and pain behavior may be explained by perivascular nerve growth or stimulation of subchondral nerves following loss of osteochondral integrity. Our data suggest that targeting angiogenesis may have utility in the treatment of pain associated with structural damage in OA.

Introduction

Osteoarthritis (OA) is a chronic and progressive disorder of the joints that is widely thought of as primarily affecting the cartilage with some bone changes. However, some authors have hypothesized that the primary disorder is in the subchondral vasculature and that the changes in the cartilage and osteophyte generation are secondary phenomena[1]. Normal adult human cartilage is both avascular[2] and insensate[3]. Angiogenesis occurs at the osteochondral junction in OA[4], where vascular channels breach the tidemark[5,6] and contain sympathetic and sensory nerves[7,8]. These findings lead to the hypothesis that angiogenesis facilitates innervation of articular cartilage, and therefore may be an important structural change that leads to pain. Matrix metalloproteinases (MMPs) are known to degrade the cartilage[9] and therefore they may have a role in the ingrowth of blood vessels and nerves during the initiation of OA. We hypothesized that inhibition of MMPs would reduce pain behavior in OA by inhibiting osteochondral angiogenesis.

The meniscal transection (MNX)[10] model of OA has been well characterized in terms of chondropathy and osteophytosis[10]. Recently, we have shown in this model that blood vessels also cross the osteochondral junction[11], comparable with observations in the anterior cruciate ligament transaction model of OA[12] and in human OA[7]. The MNX model is sensitive to inhibitors of MMPs[10] which have a protective effect on overall cartilage damage as well as reducing osteophyte formation. However, neither vascular invasion of the cartilage nor pain behavior were reported in these studies. Some protective effects are seen for MMP inhibitors in other animal models of arthritis such as monosodium iodoacetate-induced[13], adjuvant[14] and canine surgically-induced arthritides[15]. Hind paw weight-bearing assymetry is a measure of pain behavior. The MNX

* Address correspondence and reprint requests to: Paul I. Mapp, Academic Rheumatology, Clinical Sciences Building, City Hospital, Nottingham, NG5 1PB, UK. Tel: 44-(0)115-823-1758; Fax: 44-(0)115-823-1757.
E-mail address: Paul.Mapp@Nottingham.ac.uk (P.I. Mapp).

1063-4584/$ – see front matter
doi:10.1016/j.joca.2009.12.006

model induces changes in hind paw weight distribution, which were attenuated by a COX -2 inhibitor and gabapentin[16].

Associations between OA structural change and pain behavior are often weak, and are incompletely understood, both in animal models and in human disease[17–19]. In order to further explore possible mechanisms linking structural change and pain, we examined whether the oral administration of a MMP inhibitor would have an effect on joint pathology, osteochondral vascularity, and pain behavior, and explored possible interactions between any such effects.

Methods

MMP inhibitor characterization

Compound potency and Matrixin family selectivity of M503902 was determined *in vitro* using Fluorescence Resonance Emission Transfer (FRET) assays, based on the methodology previously described[20]. Briefly, compounds were solubilised in dimethyl sulphoxide (DMSO, Sigma Aldrich UK) to 10 mM and serially diluted in assay buffer, (1% (v/v) DMSO, 0.1 M Tris-HCL, 0.1 M NaCl, 10 mM $CaCl_2$, 005% (v/v) Brij 35) and 50 μl added to 96 well Black assay plates (Cliniplates, Labsystems UK) at final concentrations 0.001 nM–100μM. 25 μl of active enzyme (final concentration 0.1–0.7 mg/ml) in Zinc Calcium buffer, (0.1 M Tris-HCL pH 7.5, 0.1 M NaCl, 0.08 M $ZnCl_2$, 0.05% (w/v) Brij 35, 0.05 M $CaCl_2$) was added to the compound and incubated for 15 min on a plate shaker at room temperature prior to addition of substrate at Km, (see Table I for specific assay substrate). Plates were incubated in a humidity chamber at 35°C for up to 2 h.

Fluorescence was read at time 0 and every 30 min (Spectramax, Molecular Devices UK) at wavelengths of excitation 328 nm, emission 393 nm until a suitable window was reached. IC_{50}s were calculated using an in house automated software package.

Induction of OA

All *in vivo* procedures were carried out in accordance to the UK Animals (Scientific Procedures) Act 1986 and approved by AstraZeneca local ethical review . OA was induced by medial MNX in male Lewis rats (270 g, Harlan, Bicester, UK), as previously described[14]. Briefly, pathology was induced by transecting the medial collateral ligament and making a full thickness cut through the meniscus (day 0) of the left knee. For controls, medial collateral ligament transection without MNX (SHAM) surgery was performed on the left knee of separate animals.

Treatments

Animals (10 rats per group) were dosed twice daily by oral gavage with either vehicle (hydroxyprolyl methyl cellulose/tween) or vehicle containing the MMP inhibitor, AstraZeneca M503902 (0.125, 0.5, 2.5 mg/kg from the day prior to surgery, until sacrifice at day 35). The inhibitory profile of M503902 against eleven MMPs is given in Table I.

Pain behavior

Pain behavior was measured as weight-bearing asymmetry between operated and contralateral knees. Changes in weight-bearing asymmetry were assessed at pre surgery (−2 days) and at 7, 14, 21, 28 and 35 days post surgery, using an Incapacitance meter (Linton Instruments, Diss, Norfolk, UK)[21,22]. This technique measures the difference in weight bearing between the ipsilateral operated limb and the contralateral control limb. Rats were placed in a Perspex container such that each paw rested on a separate transducer pad that recorded the animals weight distribution over a period of 3 sec. Each data point is the average of three readings. The hind paw weight distribution is expressed as the difference in weight between ipsilateral and contralateral limbs. Weight-bearing asymmetry was analysed as area under the curve, and possible associations with histological parameters were determined using measurements obtained prior to sacrifice 35 days after surgery.

Histology

For each animal, skin was removed and the tibiofemoral joints were isolated by cutting mid-femur and tibia. The joints including articular surfaces, joint capsule and intra-articular structures were preserved in 10% neutral buffered formalin for 48–72 h and subsequently decalcified for 24–36 h in rapid decalcification fluid (Surgipath decalcifier II – Surgipath Europe Ltd, Peterborough, UK). Trimmed joint tissues were processed by standard histological techniques and mounted in wax blocks for sectioning. Coronal sections (5 μm), through the midpoint of the joint, identified by the presence of cruciate ligament insertions, were stained with toluidine blue.

Chondropathy and osteophytosis were evaluated using the system of Janusz *et al.*[10]. In this method cartilage damage was scored on a scale of 0–5 as follows: 0. Cartilage of normal appearance; 1. Minimal fibrillation, superficial zone only; 2. Mild, extends to the upper middle zone; 3. Moderate, well into the middle zone; 4. Marked, into the deep zone but not to the tidemark; and 5. Severe, full thickness degeneration to tidemark. The amount of cartilage damage was estimated as the proportion of the section of the medial tibial plateaux involved, 1/3, 2/3 or 3/3 and the cartilage score multiplied by one, two or three respectively to give a total chondropathy score. Osteophytosis was scored on a scale of 0–3, using an eyepiece graticule as follows: 0. No osteophyte present; 1. Mild, <50 μm; 2. Moderate, 50–150 μm; and 3. Severe, >150 μm.

Table I
The ability of M503902 to inhibit purified full-length recombinant human MMPs (expressed in bacculo virus) was determined against synthetic peptide substrates, (substrate concentration at Km)

Matrixin	Peptide substrate	M503902	
		IC_{50} mean ± S.E.M (n=)	Ratio (MMPx/MMP13)
MMP-13	MCA-Pro-Cha-Gly-Nva-Dpa-Ala-Arg-NH2	0.35 ± 0.05 nM[3]	1
MMP-1	MCA-Pro-Cha-Gly-Nva-His-Ala-Dpa-NH2	119 ± 14.5 nM[3]	340
MMP-2	MCA-Pro-Cha-Gly-Nva-His-Ala-Dpa-NH2	0.1 nM[1]	0.29
MMP-3	MCA-Pro-Cha-Gly-Nva-His-Ala-Dpa-NH2	1.9 nM[1]	5.4
MMP-7	MCA-Pro-Cha-Gly-Nva-His-Ala-Dpa-NH2	325 ± 28.9 nM[3]	929
MMP-8	MCA-Pro-Cha-Gly-Nva-His-Ala-Dpa-NH2	0.6 nM[1]	1.71
MMP-9	MCA-Pro-Cha-Gly-Nva-His-Ala-Dpa-NH2	0.26 ± 0.06 nM[5]	0.74
MMP-12	MCA-Pro-Cha-Gly-Nva-His-Ala-Dpa-NH2	0.45 nM[1]	1.26
MMP-14	MCA-Pro-Cha-Gly-Nva-His-Ala-Dpa-NH2	3.05 ± 0.67 nM[5]	8.7
MMP-19	MCA-Pro-Leu-Ala-Nva-Dpa-Ala-Arg-NH2	7.2 ± 0.24 nM[11]	20.6

MMP_x = X is the number of matrixin in column 1.