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Site specific changes in gene expression and cartilage metabolism during early experimental osteoarthritis

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Summary

Objectives: To characterize the molecular events underlying cartilage injury in the early phase of mono-iodoacetate-induced osteoarthritis (OA) in rats.

Methods: Experimental osteoarthritis was induced by intra-articular injection of 0.03 mg mono-iodoacetate (MIA) in Wistar rats. Animals were killed 2, 5, 10, 15 and 20 days post-injection. Synovial tissue and standardized biopsies from different areas of knee cartilage were examined. Proteoglycan synthesis (^{35}S incorporation) and gelatinase activities (zymography), semi-quantitative RT-PCR and immunohistochemistry for IL1β, iNOS, COX2 and PPARγ, were performed on these samples.

Results: Changes in proteoglycan synthesis and gelatinase activities were time and site-dependent. Proteoglycan synthesis inhibition was maximal by day 2 while the highest gelatinase activities were observed at day 5. Central part of patella and posterior plateaus and condyles, i.e. the weight-bearing cartilage areas, were the most affected.

IL1β and iNOS transcripts were induced early in cartilage at time of maximal proteoglycan synthesis inhibition, especially in weight-bearing areas. COX-2 was slightly up-regulated whereas PPARγ gene expression remained unchanged. Gene expression profile in synovium paralleled that of cartilage, except for PPARγ which was up-regulated at day 15 and 20.

Immunostaining for IL1β and iNOS showed that proteins were located in diseased cartilage areas at early stage of the experimental OA (day 2). At later time-points (day 20), IL1β and iNOS were expressed in perilesional areas whereas immunostaining became below control level for COX-2 and PPARγ.

Conclusions: Time-dependent degradation of cartilage after injection of low dose of MIA (0.03mg) into rat knee joint can be related to early loss of proteoglycan anabolism, increased gelatinase activities and expression of IL1β and downstream inflammatory genes. Increased susceptibility to MIA in weight-bearing areas of cartilage further indicate that MIA-induced experimental OA is a relevant model to study not only metabolical but also biomechanical aspects of human OA.

Key words: Experimental osteoarthritis, Rat model, Interleukin-1, Proteoglycans, Matrix metalloproteinases.

Introduction

Osteoarthritis (OA) is a degenerative joint disease which affects a large population and results in significant morbidity and disability. The clinical condition is characterized by focal cartilage destruction, concomitant hypertrophic reactions in the bone, including osteophyte formation at the joint margins, and inflammation of the synovium[1].

In an attempt to study the pathophysiology of the early phases of the disease and to identify new targets for therapeutic modalities, numerous experimental models that mimic aspects of human OA have been developed. Unlike spontaneous OA which has slow onset and probably originates in unknown genetic predisposition, experimental induction of OA in animals makes it possible to influence the onset and the course of the disease. OA can be induced by different ways, including load impact induced trauma, surgically- or enzymatically-induced joint instability, extracellular matrix damage induced by collagenase or disturbance of chondrocyte metabolism induced by mono-iodoacetate (MIA), vitamin A or steroids[2]. In mice, injection of MIA led to a strong inhibition of proteoglycan synthesis in the central part of the patella and the medial part of the tibial plateau[3,4]. Subsequently, the loss of locomotor activity, and the severity of cartilage lesions in patella were directly related to the injected dose of iodoacetate, allowing for a grading of OA severity in rats[5].

The involvement of several cytokines and enzymes has been reported in human OA. IL1β has been shown to be one of the most deleterious cytokine for cartilage *in vivo* and *in vitro*: it can inhibit proteoglycan anabolism and promote degradation of extracellular matrix components through activation of metalloproteinases (MMPs)[6–8]. The chondroprotective efficacy of IL1β receptor antagonist in animal models of OA further supports the deleterious effects of IL1β on cartilage[9,10]. IL1β can mediate its effects

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Table I

Gene (length)	Primers (forward-reverse)	Cycles	Tm
L27	5′-TCC-TGG-CTG-GAC-GCT-ACT-C-3′	25	61°C
225 bp	5′-CCA-CAG-AGT-ACC-TTG-TGG-GC-3′		
IL1β	5′-TGA-AAG-CTC-TCC-ACC-TCA-ATG-G-3′	35	55°C
367 bp	5′-TCC-ATG-GTG-AAG-TCA-ACT-ATG-TCC-3′		
COX-2	5′-TCC-AGA-TCA-CAT-TTG-ATT-GAC-AG -3′	30	59°C
443 bp	5′-TGT-GGG-AGG-ATA-CAT-CTC-TCC-3′		
iNOS	5′-AGC-AGA-ATG-TGA-CCA-TCA-TGG-ACC-3′	30	60°C
433 bp	5′-ATG-CAG-ACA-ACC-TTG-GTG-TTG-AAG-3′		
PPARγ	5′-ATA-AAG-TCC-CTT-CCC-GCT-GAC-CAA-AGC-3′	30	64°C
523 bp	5′-CGG-TCT-CCA-CTG-AGA-ATA-ATG-ACA-GC-3′		

at least through induction of COX-2 and iNOS expression, which produce the inflammatory mediators prostaglandins and nitric oxide (NO), respectively[11–15]. Indeed, NO interferes with chondrocyte functions, resulting in loss of cartilage matrix through MMPs activation, induction of apoptosis or inhibition of proteoglycan and type II collagen synthesis[16–18]. The role of PGE2 in proteoglycan degradation is more controversial: while Di Battista showed that PGE2 increased proteoglycan synthesis *in vitro*[19], IL1-induced production of PGE2 contributed to cartilage degradation in human osteoarthritic joint tissues[20]. Besides, the identification of endogenous factors which are able to modulate IL1β-dependent cartilage degradation is still under investigation. *In vitro*, activation of peroxisome proliferators activated receptor gamma (PPARγ) has been shown to counteract IL1β-induced expression of inflammatory genes or MMPs in chondrocytes and synoviocytes[21–23].

Experimental models of OA often play a pivotal role in new target discovery/validation and drug evaluation. Nevertheless, while MIA-induced effects on cartilage and especially on chondrocytes metabolism have been reported in details, the events involved in initiation and progression of this experimental OA are still poorly understood. Therefore, we designed the present study to describe the molecular processes underlying cartilage injury during the course of the MIA-induced OA, thereby providing evidence for the contribution of IL1β and its effectors (iNOS, COX2) and PPARγ in the early phase of this model. Since focal erosions have been documented in human, it remains to examine to what extent different sites within the same joints are affected in a course of a chemically induced OA. Thus, we attempted to relate variations in gene expression to regional differences in MMPs activity and proteoglycan synthesis.

Materials and methods

ANIMALS

Male Wistar rats (150–175 g), obtained from Charles River (L'arbresle, France) were housed under controlled temperature and lighting conditions with food and water *ad libitum*. Animals were acclimatized to the laboratory environment for one week before experiment. Our local Animal Care and Use Committee approved the experimental protocols, and guidelines for laboratory procedures were followed at all times.

EXPERIMENTAL DESIGN

Rats were injected in both knee joints with MIA (0.03 mg/knee) (Sigma St Quentin Fallavier, France) in a volume of 50 µl sterile saline. Rats injected with saline alone served as controls. At day 2, 5, 10, 15, and 20 post-injection, synovia, patellae, femoral condyles and tibial plateaus were collected. Synovia were immediately frozen at –80°C for RNA extraction and measurement of MMPs activity. Patellae, femoral condyles and tibial plateaus were processed for *ex vivo* incorporation of $Na^{35}SO_4$ into cartilage, RNA extraction, immunohistochemical analysis or measure of MMP activity.

HISTOLOGICAL STUDY

Knee joints from saline and MIA-injected rats were fixed for 24 h in 4% paraformaldehyde immediately after sacrifice, decalcified in rapid bone decalcifier (RDO, Apex engineering, Plainfield, U.S.A.) for 4 h, further fixed in 4% paraformaldehyde and embedded in paraffin. Sections (5 µm thick) of femoro-tibial joints were rehydrated in a graded series of ethanol and stained for collagen and proteoglycan with Safranin-O-Fast-Green.

PROTEOGLYCAN SYNTHESIS

Cartilage explants – *i. e.* patellae, femoral condyles and tibial plateaus-were placed for 3 h at 37°C in a humidified 5% CO_2 atmosphere with 0.6 µCi/ml $Na_2^{35}SO_4$ (Amersham, Les Ulis, France) in RPMI Hepes 1640 medium supplemented with 2 mM L-glutamine, 100 µg/ml streptomycin, and 100 IU/ml penicillin. After 5 washings in saline, explants were fixed overnight at room temperature in 0.5% cetylpyridinium chloride dissolved in 10% formalin buffer. Explants were then decalcified for 6 h in 5% formic acid, and biopsies (2 mm diameter) were punched out from the surrounding tissues. Biopsies were dissolved in 0.5 ml Soluene (Packard, Rungis, France) overnight at 42°C. The ^{35}S content of each individual biopsy was measured by liquid scintillation counting (Packard, Rungis, France).

GELATIN ZYMOGRAPHY

Dissected intact cartilage was immediately homogenized in 0.5 ml homogenization buffer (50 mM Tris HCl, pH 7.4, 300 mM KCl, and 2.5 mM $MgCl_2$) using Polytron PT (Kinematica, Luzern, Switzerland). Aliquots (10 µl) of cartilage homogenates containing 0.25–2 µg total protein were mixed with nonreducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis sample buffer and electrophoresed in 10% polyacrylamide gels containing 1 mg/ml type A gelatin from porcine skin, at 20 mA constant current for 1.5–2 h at 4°C. Following electrophoresis, gels

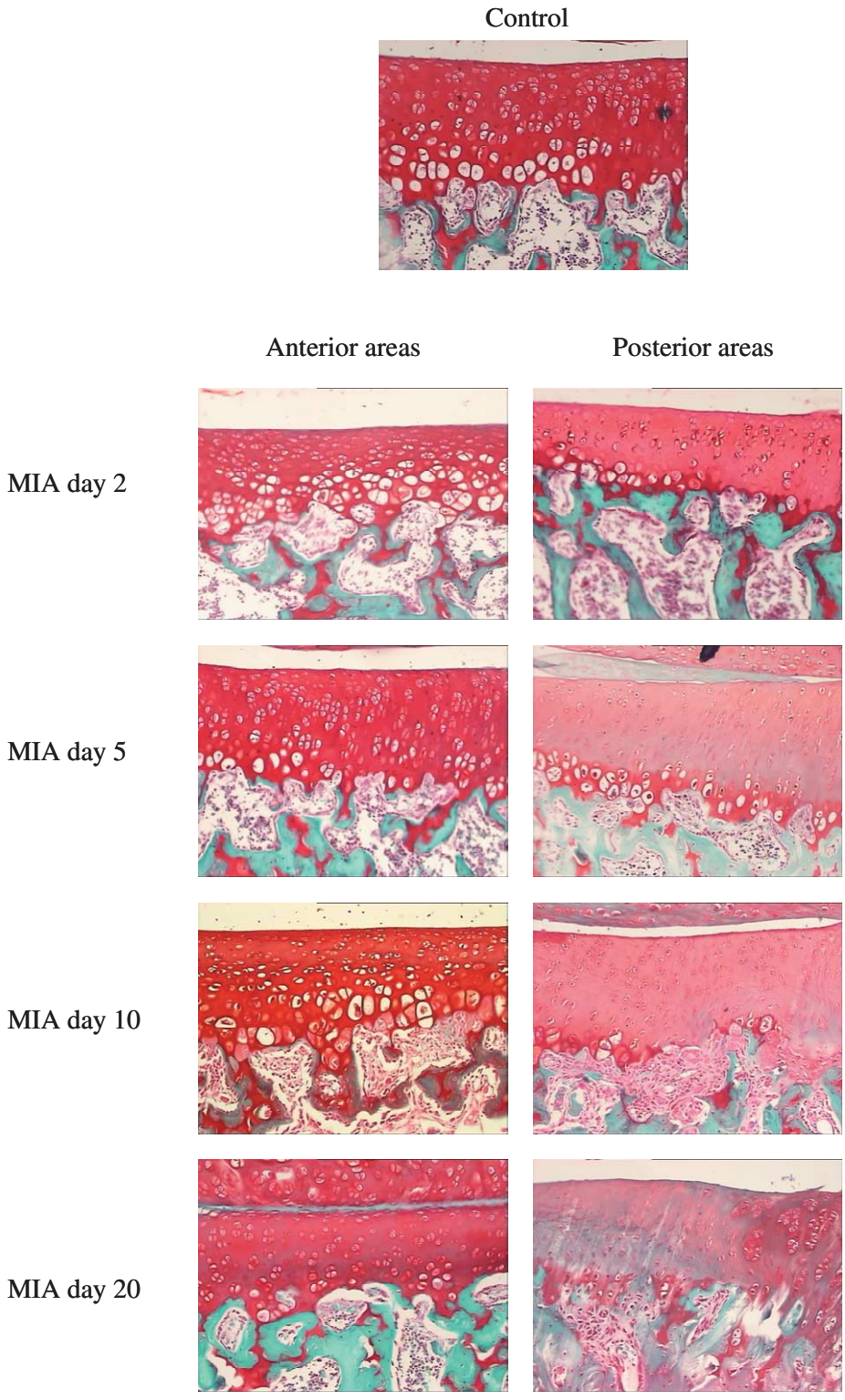

Fig. 1. Effects of MIA on cartilage matrix. Safranin-O-fast-green staining on representative sections of tibial plateaus from rats killed at day 2, 5, 10 and 20 after 0.03 mg MIA injection. Original magnification ×40.

were washed twice for 20 min with 2.5% Triton X-100 in Tris buffered saline (50 mM Tris HCl, pH 7.5, 150 mM NaCl) to allow protein to renature. Gels were then incubated 20–40 h at 37°C in substrate buffer (50 mM Tris HCl, pH 8, 10 mM $CaCl_2$, and 0.02% NaN_3), stained with Coomassie R250 for 30 min and destained for 1 h. Gelatin-degrading enzymes were visualized as clear bands, indicating proteolysis of the substrate protein. Gels were scanned and

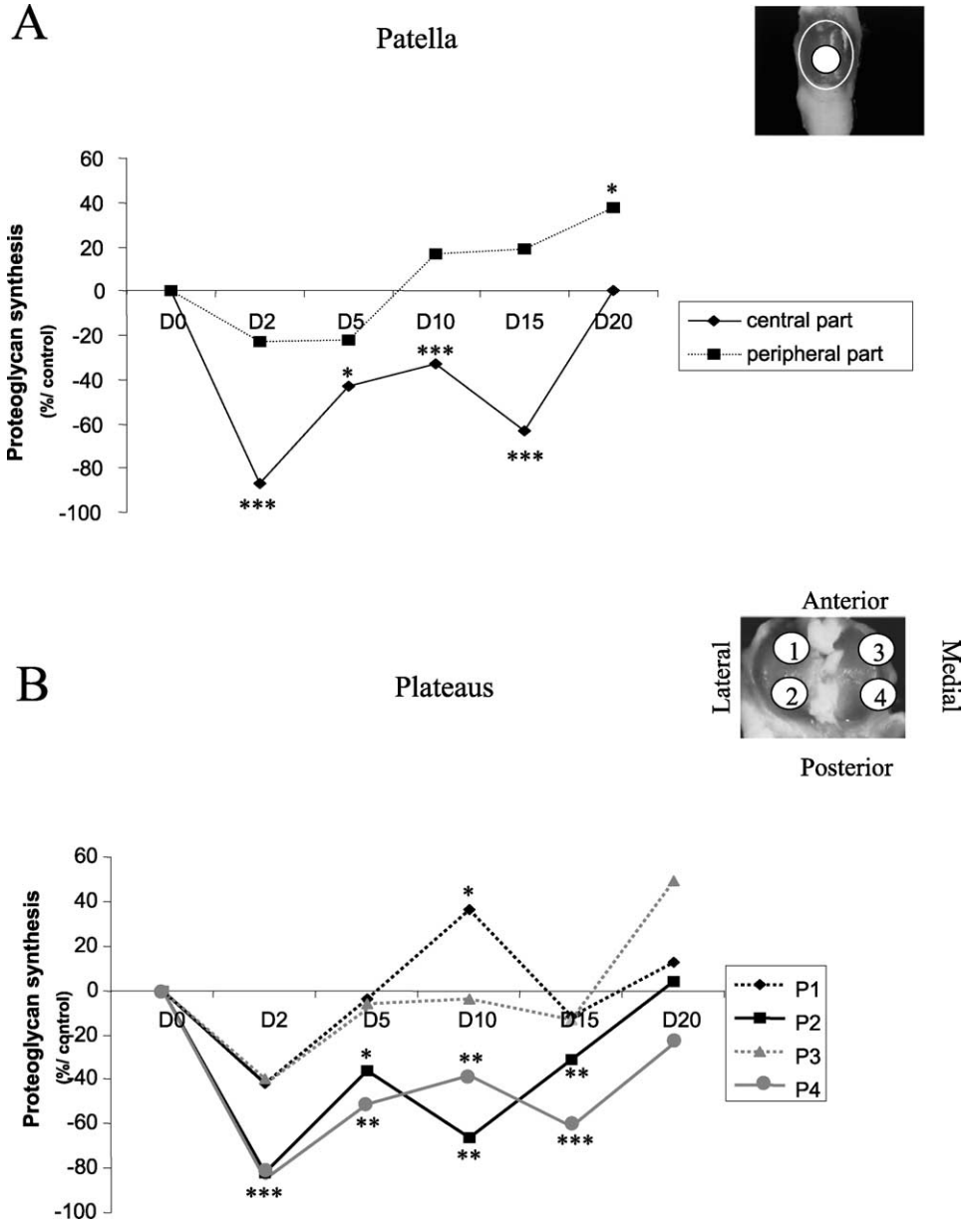

Fig. 2. A and B.

Fig. 2. Time-course of proteoglycan synthesis in cartilage biopsies following MIA administration. Proteoglycan synthesis was measured by $^{35}SO_4^{2-}$ incorporation in standardized biopsies of cartilage (localization indicated in the corresponding scheme) after intra-articular injection of 0.03 mg MIA (patella (A), plateaus (B), femoral groove (C) and condyles (D)). Results are expressed as the percentage of ^{35}S incorporation in cartilage from MIA-injected rats vs control animals (N=6). (*=P<0.05; **=P<0.01; ***=P<0.001).

analyzed by a Viber Lourmat (Torey, France) imaging system. Molecular weight markers (Bio-Rad) and positive controls, human MMP-2 and MMP-9 (Oncogen Research Products, Boston, MA) were used in all gels.

REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION (RT-PCR)

Explants were decalcified for 18–24 h with EDTA 165 mM pH=7.4 in RNA later (Ambion, Huntingdon, U.K.) before separation of the cartilage layer from the underlying bone. Total RNAs from synovium and cartilage were isolated using a commercially available phenol-chloroform solution (Trizol) (Sigma, St Quentin Fallavier, France). Integrity of RNA pool was verified by electrophoresis. mRNAs (2 µg) were reverse transcribed for 1.5 h at 37°C using M-MLV reverse transcriptase (200 U) (Gibco BRL, Cergy Pontoise, France) and anchored 15 mer oligo-dT primers (100 pmol) (MWG biotech SA, Courtaboeuf, France). PCR amplification was then performed with 1/10th of RT products with Taq polymerase (2.5 U) (Gibco BRL, Cergy Pontoise, France) using specific primers (MWG biotech SA, Courtaboeuf, France). The conditions for amplification were: denaturation at 94°C for 45 s, hybridization of primers at defined temperature for 45 s and elongation at 72°C for 45 s (see Table I).

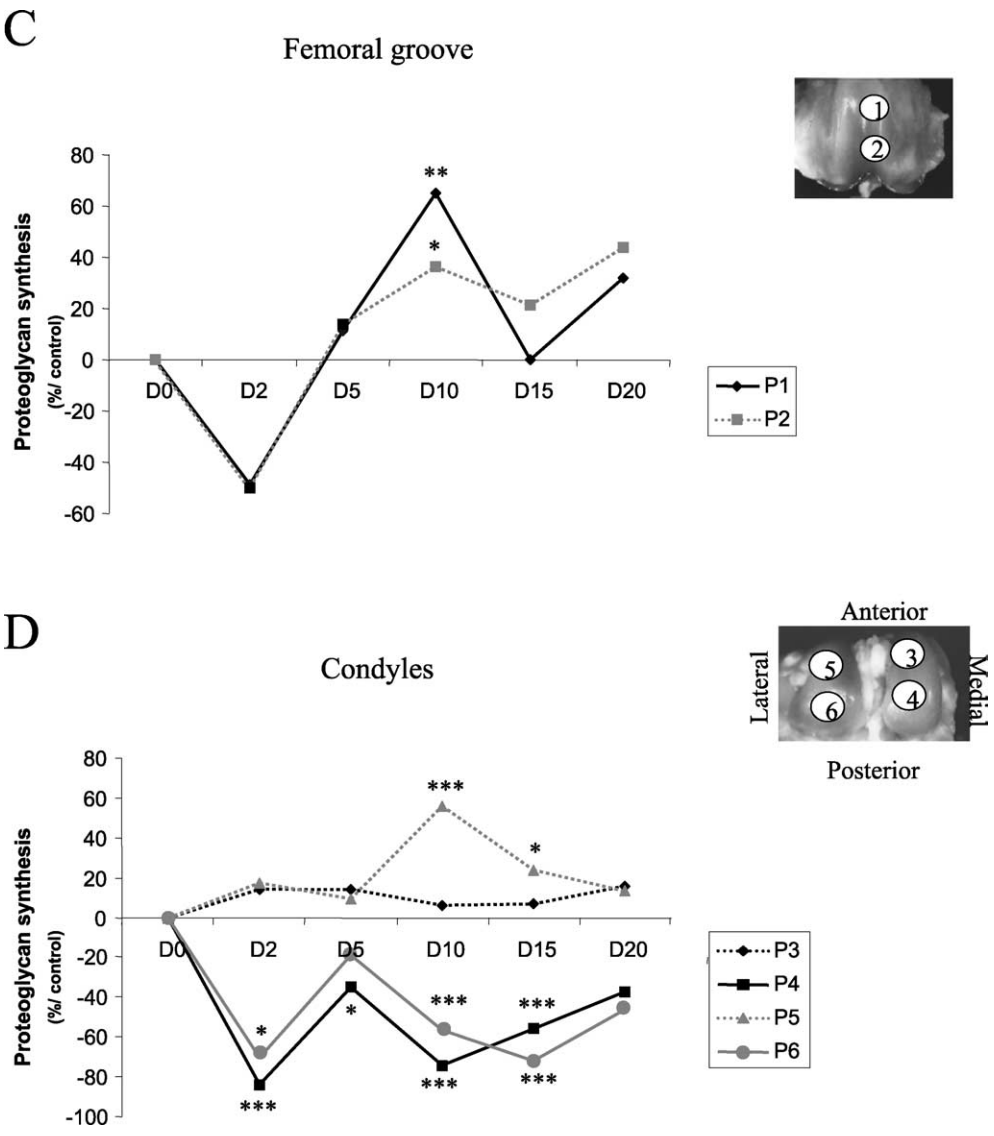

Fig. 2. C and D

As an internal control, the same RNA preparation was also subjected to RT-PCR analysis for the housekeeping gene L27, encoding a ribosomal protein. The PCR products were analyzed by 0.2 µg/ml ethidium bromide staining in 1.5% agarose gel. Quantification was performed with Geldoc 2000 software (Biorad, Marnes-la-Coquette, France).

IMMUNOHISTOCHEMICAL ANALYSIS

Femoral condyles and tibial plateaus were fixed in 4% paraformaldehyde for at least 24 h, decalcified for 4 h in RDO, further fixed for 24 h in paraformaldehyde, and then embedded in paraffin. Paraffin sections (5 µm thick) were deparaffinized in toluene, and hydrated in a graded series of ethanol. Slices were incubated subsequently in BSA 4% as blocking reagent for at least 2 h, then overnight at 4°C with a polyclonal primary antibody (COX-2, 10 µg/ml, sc-7951 Santa-Cruz; iNOS, 2 µg/ml, sc-650 Santa-Cruz; IL1β, 6 µg/ml, sc-7884 Santa-Cruz; PPARγ, 6.7 µg/ml, sc-7196 Santa-Cruz) (Tebu, Le Perray-en-Yvelines, France) and finally with biotinylated secondary antibody (1 µg/ml) for 45 min at room temperature. Signal was then amplified with preformed avidin-biotinylated horseradish peroxidase complexes for 45 min at room temperature and, staining was developed with 3,3′-diaminobenzidine (0.05% in hydrogen peroxide) (Novostain super ABC kit, Novocastra, Kington, U.K.).

STATISTICAL ANALYSIS

For RT-PCR analysis, each value is a mean of at least four animals (both knees from one animal being pooled in one sample). The RT-PCR analysis were repeated twice for one set of animals. Six samples for the determination of proteoglycan synthesis were analyzed from three independent experiments. The results was analyzed by ANOVA, and P values less than 0.05 were considered significant (*=P<0.05; **=P<0.01; ***=P<0.001).

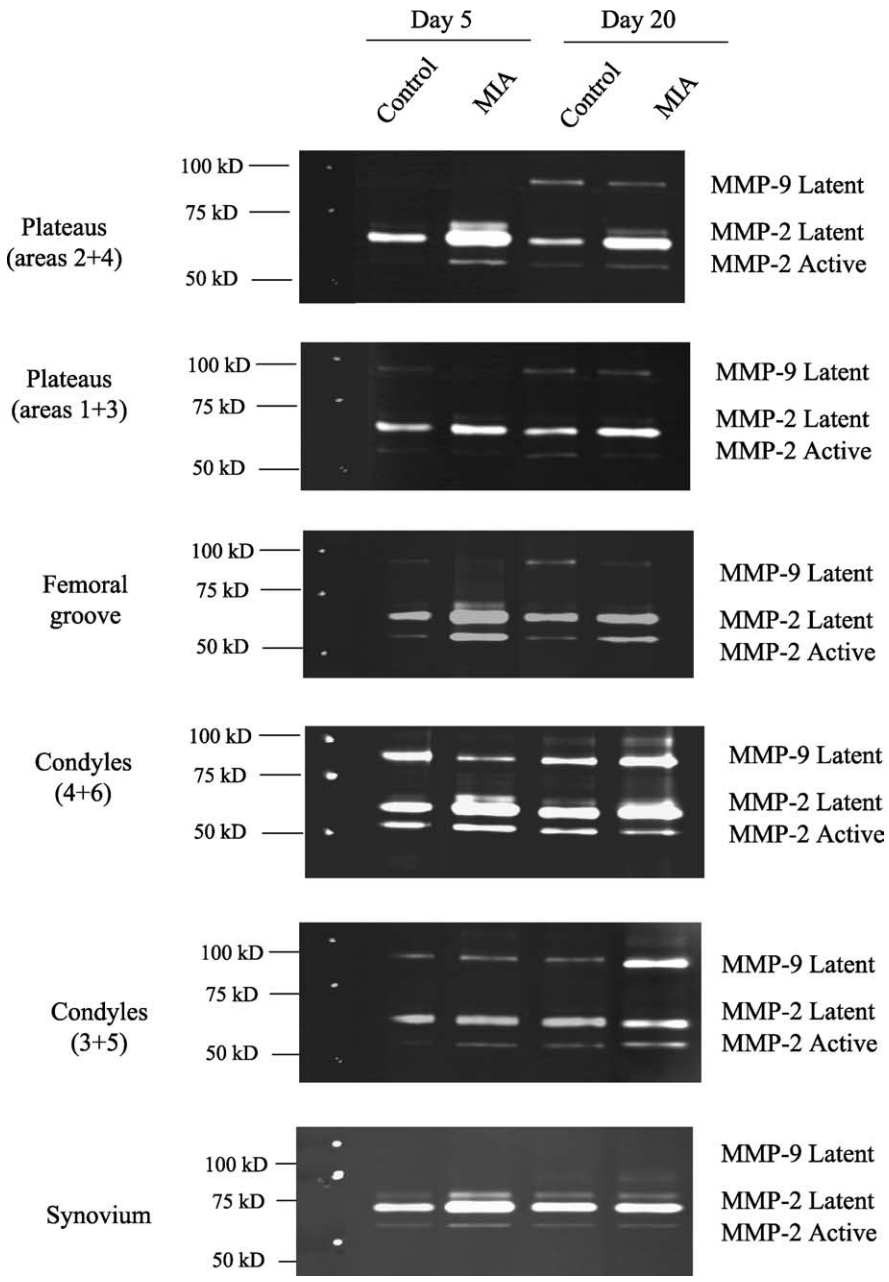

Fig. 3. Gelatinase activities assessed by zymography in knee joint cartilage and synovium homogenates prepared from tibial plateaus, femoral condyles and synovium obtained 5 or 20 days after injection of 0.03 mg MIA.

Results

MIA INDUCES HISTOLOGICAL MODIFICATIONS IN RAT ARTICULAR CARTILAGE

A single injection of a low dose of MIA (0.03 mg) induced cartilage lesions in the posterior (severe in central part and moderate at the margin) but not in the anterior part of the tibial plateaus (Fig. 1). In comparison to saline–injected controls, early changes included matrix depletion in superficial layer by day 2 and a rarefaction of chondrocytes in primarily affected areas. By day 5, proteoglycan loss spread out to the deep zone of the tibial plateaus and appeared in the superficial layers of the femoral condyle. From day 10 to day 20 of the time-course, hypertrophic chondrocytes disappeared, the width of the lesion increased, as well as the number of necrotic chondrocytes, except in the lateral part of the articular surface. By day 20, proteoglycan depletion was enhanced with fibrillation of the cartilage surface and some subchondral bone sclerosis in few animals.

MIA-INDUCED VARIATIONS OF PROTEOGLYCAN SYNTHESIS ARE TIME- AND SITE-DEPENDENT

Proteoglycan synthesis, assessed by *ex vivo* incorporation of radiolabeled sulfate into cartilage, was examined in cartilage biopsies from different areas of the knee joint (Fig. 2), allowing to distinguish central/peripheral parts of

the patella, anterior/posterior and lateral/medial parts of tibial plateaus and femoral condyles. MIA injection induced time- and site-dependent changes in articular cartilage: strong biphasic variations of proteoglycan synthesis were observed in central patella, femoral groove, posterior plateaus and condyles while they were less intense in peripheral patella, anterior plateaus and anterior condyles.

In patella, a strong inhibition of ^{35}S incorporation was observed at day 2, especially in the central part, and was followed by a transient recovery at day 5 and day 10. However, proteoglycan synthesis decreased again at day 15 and returned to basal level at day 20. No significant reduction in cartilage anabolism was also noted in the peripheral part (day 2 and day 5), but proteoglycan synthesis rate remained stable over control level at later time points (Fig. 2A).

In tibial plateaus (Fig. 2B), proteoglycan synthesis was strongly inhibited in posterior parts (P2 and P4) from day 2 (more than 80%) to day 15, with recovery at day 20. In the anterior areas (P1 and P3), proteoglycan synthesis was less inhibited (20 to 40%) at day 2, returned to control level at day 5 and tended to overcome basal level at later time points.

In femoral groove, ^{35}S incorporation was inhibited by about 50% at day 2 only. An overshoot ranging from 38% to 65% was observed at day 10 (Fig. 2C). As for tibial plateaus, proteoglycan synthesis varied depending on the site of femoral condyles (Fig. 2D) with inhibition restricted to the posterior areas (biopsies 4 and 6), although recovery remained incomplete at day 20.

MIA-INDUCED GELATINASE ACTIVITIES ARE TIME- AND SITE-DEPENDENT

Gelatin zymography of each cartilage area (patella, tibial plateaus, femoral groove, femoral condyles) exhibited time and site-dependent variation in the activity of MMP-2 (latent form 72 kD, active form 66 kD) and MMP-9 (latent form 97 kD) at day 2, 5, 10, 15 and 20 following MIA injection. In synovium, MMP-9 activity was not detected, and only minor changes in MMP-2 were observed between samples from MIA-injected rats and controls (Fig. 3).

In tibial plateaus, an increase in MMP-2 (latent form) was detected in posterior areas (biopsies 2 and 4) and, to a lesser extend in anterior ones (biopsies 1 and 3) at day 5 and day 20 (Fig. 3). However, an increase was observed for the active form in the posterior areas.

In femoral groove and posterior condyles, an increase in MMP-2 activity was measured whatever the time-point, whereas there were no significant differences in anterior condyles. MMP-9 activity did not vary between cartilage from MIA-injected rats and controls, except at day 20 in anterior condyles (Fig. 3).

In patella, no variation of gelatinase activity was observed in the course of the model (data not shown).

IL1β, COX2, iNOS, PPARγ mRNAs ARE EXPRESSED IN THE EARLY PHASE OF MIA-INDUCED OA

As shown in Fig. 4, IL1β expression increased significantly at day 2 in patella and synovium (6-fold and 2.7-fold, respectively). A 2-fold increase was observed at day 2 for COX-2 mRNA in both tissues, and for PPARγ mRNA in patella only (Fig. 4A). At day 5, genes expression returned to control levels except for PPARγ in synovium, for which expression increased strongly at day 5 and 10 (Fig. 4B).

No significant variation in mRNAs expression was detected in the overall plateaus or condyles (data not shown). However, by separating the different cartilage areas, we were able to detect at day 2 a strong increase in IL1β expression (2 to 4.8-fold) in posterior plateaus (Fig. 5A), and an induction of IL1β and iNOS in femoral groove (Fig. 5B). Expression levels in anterior areas of plateaus and in condyles did not differ from control levels (Fig. 5A and 5B). The level of PPARγ expression was too weak to detect any significant variation in these biopsies.

IL1β, COX2, iNOS, PPARγ PROTEINS ARE EXPRESSED DURING MIA-INDUCED OA

Immunohistochemical analysis of serial sections showed that IL1β, COX2, iNOS and PPARγ were expressed in condyles and plateaus from control rats (Fig. 6). Two days after MIA injection, expression of iNOS, COX2 and IL1β was enhanced in most chondrocytes from the superficial layer of cartilage. At day 5, 10, 15 and 20, IL1β, iNOS and COX2 expressing chondrocytes were located at the periphery of the lesions whereas staining decreased progressively until disappearance in diseased areas. Basal expression of PPARγ was down-regulated in MIA-treated rats from day 2 to day 20 despite a transient and moderate immunoreactivity at day 15.

Discussion

In this study, we determined the time-course of cartilage changes within various parts of the knee joint in the MIA-induced experimental OA in rat. We observed that loss of proteoglycan anabolism occurred early in the disease in specific cartilage areas but lessened at later time points: proteoglycan synthesis was back to control levels at day 20 whatever the biopsy tested. Besides, chondrocytes exhibited enhanced gelatinase activities from day 2 in our model and a peak of gelatinase activities was measured at day 5. This suggests that the early phase of MIA-induced OA is not only characterized by a decrease in the biosynthetic activity of cartilage but also by an increase in catabolic activities. At later time-points, positive immunostaining for MMP-2, MMP-9 and MMP-3 (stromelysin 1) was observed at the margins of the lesions (data not shown), and gelatinase activities remain detectable during the whole course of the disease, demonstrating a long-standing catabolic process in cartilage. These results are in agreement with previous study from Janusz[24] who showed that collagenase and gelatinase activities increased in the knee cartilage of rats injected with 0.25 mg MIA. Therefore, a low dose of MIA with respect to previous dose-response study[5] seems to be sufficient to induce matrix degrading enzymes and to investigate the molecular events leading to OA lesions.

Describing the molecular events underlying changes in cartilage metabolism, we showed that chondrocytes in posterior areas, which were the most sensitive to the disturbing effect of MIA on chondrocytes functions, expressed pro-inflammatory genes (IL1β, iNOS, COX2) very early in the disease. Noteworthy, their kinetic of expression varied concomitantly to changes in cartilage anabolism, since maximal expression was observed at time corresponding to maximal loss of proteoglycan synthesis. In addition, immunohistochemical analysis indicated that IL1β, COX-2 and iNOS were expressed in superficial layers of cartilage. These layers are known to be very responsive

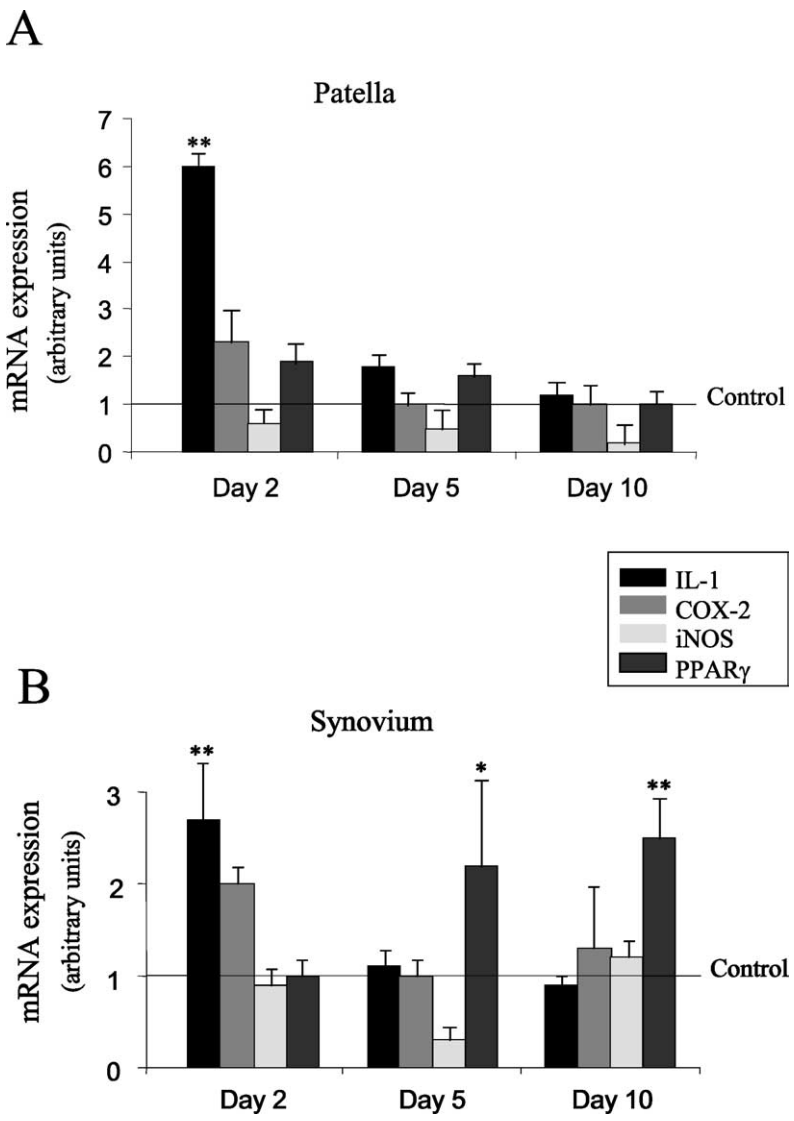

Fig. 4. Kinetics for inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX2), interleukin-1 beta (IL1β), peroxysome proliferator activated receptor gamma (PPARγ) messenger RNA (mRNA) expression in synovium and cartilage from the whole patella of rats injected with 0.03 mg MIA. After extraction from tissues collected at various time-points, total RNA was subjected to RT-PCR analysis using specific primers for each gene. PCR amplification of L27 was performed on the same RNA preparation as an internal control for the relative amounts of template. Experiments were performed in duplicate with at least four animals for each time-point. Results are expressed in arbitrary units relative to control samples±SEM (*=P<0.05; **=P<0.01).

to the deleterious effect of IL1 on cartilage[25]. Taken together, these results demonstrate that the expression of typical factors involved in human OA such as IL1β, COX2, iNOS and MMPs paralleled the initiation and progression of cartilage lesions during the MIA-induced experimental OA.

Previous *in vitro* study demonstrated that exposure to IL1β decreased mRNA expression of PPARγ in articular cells[21]. In the MIA model, we observed a lack of PPARγ protein expression and we failed to show any down regulation of PPARγ transcription in the articular cartilage, whatever the area tested. This could be due to a very low level of basal expression of the gene in rat cartilage which does not preclude that induction of PPARγ activity by synthetic ligands could have beneficial effects in articular arthropathy. By contrast, a two- to three-fold increase in PPARγ expression was observed in the synovium at day 5 and day 10 following MIA injection. Since natural ligands of PPARγ are supposed to be peculiar prostaglandins[26], previous induction of COX-2 might provide endogenous ligands to the newly synthesized receptors and might contribute to a limitation of synovial inflammation[27]. This suggests that inhibition of COX-2 activity would improve pain and acute inflammation, but could affect some endogenous control of joint homeostasis. This might explain, at least in part, the failure of NSAIDs to limit cartilage destruction in the MIA model (unpublished data).

In order to take into account the focal nature of OA lesions, we performed histological staining in anterior or posterior areas of the femoro-patellar joint, and examined proteoglycan anabolism and gelatinase activities in biopsies from several locations within the same joint. The evaluation of these three parameters showed that cartilage

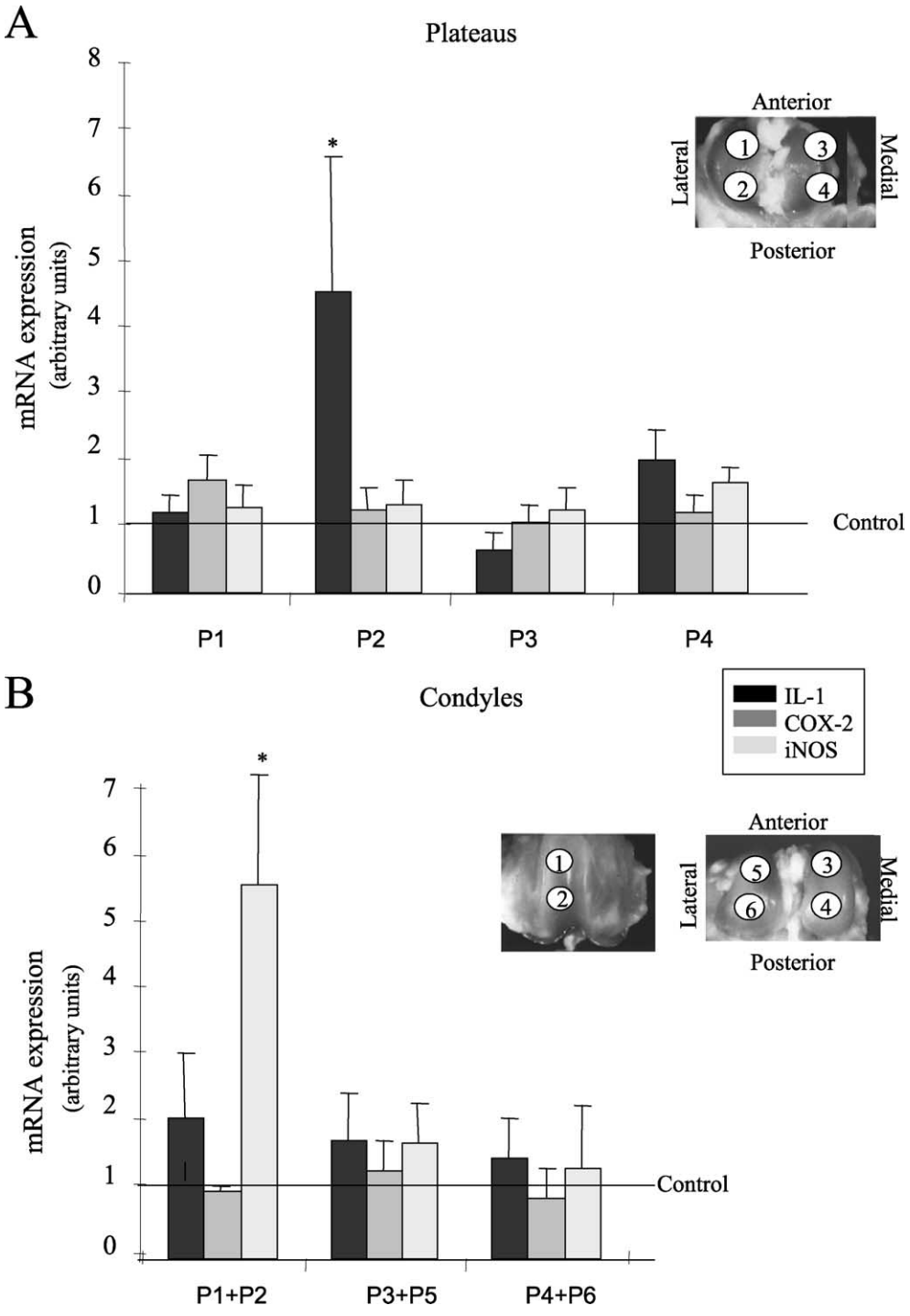

Fig. 5. Relative levels of IL1, iNOS, COX-2 and PPARγ mRNA expression in biopsies collected from plateaus (A) and condyles (B), 2 days following 0.03 mg MIA injection. PCR amplification of L27 was performed on the same RNA preparation as an internal control for the relative amounts of template. Experiments were performed in duplicate with four animals. Results are expressed in arbitrary units relative to control samples±SEM (*=P<0.05).

lesions occurred in the posterior parts of plateaus and, except for gelatinase activities, in the posterior parts of the condyles and in the central part of the patella. Because of the flexed position of rats hindpaws, central patella together with posterior plateaus and condyles correspond to weight-bearing areas of the knee joint. Therefore, susceptibility to MIA would depend on biomechanical forces heterogeneously applied to cartilage and result in focal lesions[4]. There is significant evidence that chondrocytes respond to changes in their shape and volume by regulating their metabolic activity and gene expression. These results emphasize the importance of using cartilage

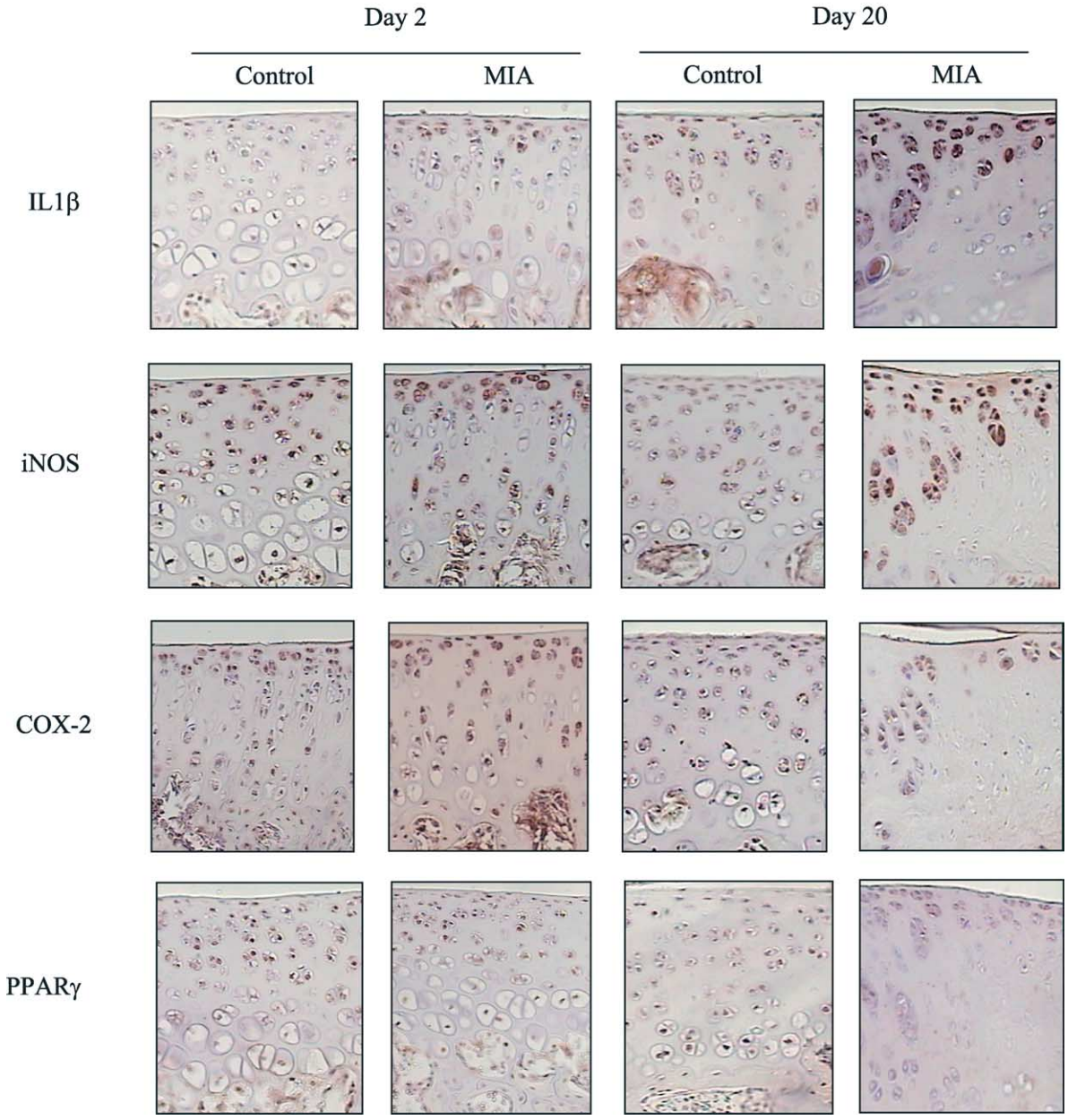

Fig. 6. IL1β, iNOS, COX-2 and PPARγ proteins 2 or 20 days after intra-articular injection of 0.03 mg MIA. Immunohistochemistry was performed on representative sections of femoro-tibial joints from MIA-injected and control rats. Original magnification ×100.

from identical joint areas when examining both proteoglycan metabolism and gene expression in chondrocytes. The relationship between mechanical stress and the incidence of OA is not clear *in vivo*, although Fermor *et al.* [28] showed recently that mechanical compression increased significantly NO and PGE2 production through a NO-dependent pathway. Therefore, we propose that MIA might first impair the function of the most metabolically active chondrocytes which are located mainly in weight-bearing areas, through inhibition of glycolysis. As a consequence, chondrocytes

may respond by increasing the production of iNOS, IL1β, COX2. Additionally, our data suggest that the expression of IL1β in these weight-bearing areas may result in MMPs activation and matrix degradation. Thus, injection of low dose of MIA points out loading contribution to OA pathophysiology and mimics the mechanical component of human OA. Although the driving role of abnormal mechanical forces has been well described in experimental OA induced by instability of the joint[29], their pathophysiological contribution to a metabolically-induced disease is described here in details. Therefore, characterization of MIA-induced OA provides information on both chondrocytes metabolism and possibly joint biomechanics.

Since MIA injection induces IL1β expression, site-dependent changes in cartilage may also result from heterogeneous distribution of cytokine receptors in articular cartilage. Recently, Westacott *et al.* showed that loading induces TNFRI but not TNFRII expression at the membrane of alginate encapsulated chondrocytes isolated from OA patients[30]. In human samples, the potential for TNFα-induced loss of cartilage is also enhanced near the articular surface of weight bearing zones, where many receptors are located[31,32]. Although no relationship between IL1RI or IL1RII expression and site sensitivity was observed for IL1β, Shlopov *et al.* [33] showed that chondrocytes located around OA lesions have a greater IL1-binding capacity than those located at more distal sites, and significant regional variation in sensitivity to IL1β was observed within the same OA femoral condyles[34]. Since early expression of IL1β, COX2 and iNOS were found in the same location as cartilage lesions in weight bearing areas, our results suggest that a combination of mechanical and MIA-mediated metabolic stress induces degrading mediators, which, in turn, initiate focal lesions. Once OA has begun, one may hypothesize that loading and pressure applied on altered cartilage could also modify locally the expression or distribution of pro-inflammatory cytokine receptors and therefore enhance chondrocyte response to these cytokines (TNFα, IL1β . . .) and their effectors (iNOS, COX2 . . .).

Human OA is often not diagnosed until the disease has reached an advanced stage, when the joints become functionally impaired and painful. Therefore, animal models provide a unique way of understanding the early events of the pathology and evaluating new therapeutic targets. In this study, we have shown that OA-like arthropathy induced by a low dose of MIA mimics the focal character of human OA. We also showed that focal lesions seem to originate in site-specific induction of pro-inflammatory factors and metalloproteinases. Since local IL1 induction appears to be the first molecular event following MIA injection, the next step of the study should be the use of an *in vivo* anti-IL1 strategy.

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