BIOCHEMICAL PHARMACOLOGY 74 (2007) 1636–1641

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/biochempharm

Requirement for protein kinase R in interleukin-1α-stimulated effects in cartilage

Christine L. Tam [a], *Maria Hofbauer* [a], *Christine A. Towle* [a,b,*]

[a] *Department of Orthopaedic Surgery, Massachusetts General Hospital, Boston, MA, United States*
[b] *Department of Orthopaedic Surgery, Harvard Medical School, Boston, MA, United States*

ARTICLE INFO

Article history:
Received 12 June 2007
Accepted 2 August 2007

Keywords:
Cartilage
Interleukin-1
Protein kinase R
Degradation
Cyclooxygenase-2
Eukaryotic initiation factor 2

ABSTRACT

Interleukin-1 (IL-1) has pleiotropic effects in cartilage. The interferon-induced, double stranded RNA-activated protein kinase PKR that phosphorylates eukaryotic initiation factor 2 (eIF2) α has been implicated in cytokine effects in chondrocytes. A compound was recently identified that potently suppresses PKR autophosphorylation (IC50 approximately 200 ηM) and partially restores PKR-inhibited translation in a cell-free system with significant effect in the nanomolar range. The objectives of this study were to exploit this potent PKR inhibitor to assess whether PKR kinase activity is required for catabolic and proinflammatory effects of IL-1α in cartilage and to determine whether IL-1α causes an increase in eIF2α phosphorylation that is antagonized by the PKR inhibitor. Cartilage explants were incubated with the PKR inhibitor and IL-1α. Culture media were assessed for sulfated glycosaminoglycan as an indicator of proteoglycan degradation and for prostaglandin E_2. Cartilage extracts were analyzed by Western blot for cyclooxygenase-2 and phosphorylated signaling molecules. Nanomolar concentrations of the PKR inhibitor suppressed proteoglycan degradation and cyclooxygenase-2 accumulation in IL-1α-activated cartilage. The PKR inhibitor stimulated or inhibited PGE_2 production with a biphasic dose response relationship. IL-1α increased the phosphorylation of both PKR and eIF2α, and nanomolar concentrations of PKR inhibitor suppressed the IL-1α-induced changes in phosphorylation. The results strongly support PKR involvement in pathways activated by IL-1α in chondrocytes.

1. Introduction

Articular cartilage is the resilient connective tissue covering the ends of bones within joints, enabling load bearing and mobility [1]. Interleukin-1 (IL-1) has pleiotropic effects on cartilage, activating proinflammatory and catabolic pathways and inhibiting the synthesis of specialized extracellular matrix macromolecules that are essential for biomechanical function. IL-1 is among the most studied cytokine regulators of cartilage metabolism, and IL-1 and other cytokines, such as tumor necrosis factor α, have been implicated in pathological changes to the cartilage in degenerative joint diseases [2,3]. IL-1 activates mitogen- and stress-activated protein kinases and transcription factors, such as nuclear factor κB (NF-κB) and AP-1 [4,5], but the signal transduction pathways linking IL-1's interaction with cell surface receptors to the diverse metabolic effects in cartilage have not been thoroughly defined.

In response to diverse cellular stresses, phosphorylation of the key translational factor eukaryotic initiation factor 2 (eIF2) on serine 51 of the α subunit causes a global down regulation of protein synthesis in mammalian cells. Four protein kinases catalyze the specific phosphorylation of eIF2α, including the

* *Corresponding author* at: GRJ 1108, Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114-2696, USA. Tel.: +1 617 724 3744; fax: +1 617 724 7396.
E-mail address: catowle@post.harvard.edu (C.A. Towle).
0006-2952/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2007.08.002

endoplasmic reticulum stress-activated kinase PERK and the double stranded RNA-activated protein kinase R (PKR). Accumulating evidence supports diverse roles for PKR in regulating cellular functions, including anti-viral response, apoptosis, cell stress response, cell growth and differentiation, cytokine signaling and inflammation [6–8]. PKR effects are mediated by eIF2 and other direct or indirect targets of PKR, including NF-κB, activating transcription factor, and signal transducers and activators of transcription (STATs).

PKR is constitutively expressed at low levels in chondrocytes, as well as in a variety of other cell types. Binding of PKR to double stranded RNA or a protein activator results in its autophosphorylation, dimerization, and kinase activity. One of the known PKR-activating proteins is PACT [9], which is overexpressed and hyperphosphorylated in tissues of aging mice [10] and overexpressed in susceptible regions of cartilage in a spontaneous model of osteoarthritis [11]. Co-stimulation of bovine articular cartilage with TNFα and IL-1α causes a prolonged increase in the phosphorylation of PKR [12]. By immunochemical techniques, specifically phosphorylated eIF2α was detected in chondrocytes 3 h after TNFα stimulation [12]. Treatment of immortalized chondrocytes of the C28/I2 cell line with IL-1β resulted in the prolonged phosphorylation of eIF2α, postulated to be part of the ER stress response catalyzed by the kinase PERK [13]. The PKR inhibitor 2-aminopurine blocked TNFα and C2-ceramide-stimulated cartilage degradation, chondrocyte apoptosis, and secretion and activation of matrix metalloproteases; however, only high concentrations that are known to inhibit other protein kinases were effective [14–16].

These results suggest that PKR may play a role in pathways associated with tissue destruction in arthritis; therefore, it is crucial to better understand the mechanisms and consequences of PKR activation in articular cartilage. Cytokines have been implicated in the pathogenesis of arthritis, with evidence from in vivo antagonist studies pointing to IL-1 as a major culprit in cartilage degeneration in rheumatoid arthritis and osteoarthritis [2,3]. The objectives of this study were to exploit a potent inhibitor of PKR to assess the necessity of this kinase for IL-1α-stimulated catabolic and proinflammatory effects in cartilage and to determine whether IL-1α causes an increase in eIF2α phosphorylation that is antagonized by the PKR inhibitor.

2. Materials and methods

2.1. Materials

Human recombinant IL-1α was a gift from the Biological Resources Branch of National Cancer Institute. The PKR inhibitor (catalogue #527450) was from EMD Biosciences, La Jolla, CA. Monoclonal antibodies to human COX-1 and COX-2 were from Cayman Chemical, Ann Arbor, MI. Antibodies to β-actin were from Sigma, St. Louis, MO. Antibodies to phosphorylation-specific and total eIF2α were from Cell Signaling Technology, Beverly, MA. Phospho-specific antibody to PKR phosphorylated on threonine 451 of the human sequence was purchased from Biosource International, Inc., Camarillo, CA. Dimethyl methylene blue was from Serva Biochemical, Heidelberg, Germany or Polysciences, Warrington, PA. Dulbecco's modified Eagle's medium, ITS supplement, and antibiotics were from Mediatech, Herndon, GA. Complete Protease Inhibitor Cocktail Tablets were from Roche Molecular Biochemicals, Mannheim, Germany. Phosphatase Inhibitor Cocktail 2 was from Sigma.

2.2. Cartilage explant culture

Cartilage cores (4-mm diameter) were taken from radiocarpal joints of one- to two-week-old calves, and 1-mm-thick disks were cut from the articular surfaces. The disks of cartilage were washed and placed in serum-free Dulbecco's modified Eagle's medium containing 5 μg/ml human recombinant insulin, 5 μg/ml human transferrin, 5 ηg/ml selenious acid, 100 U/ml penicillin and 100 μg/ml streptomycin (DMEM). Prior to initiating the experiments, disks were equilibrated to culture conditions (5% CO_2, 95% humidified air 37 °C) in DMEM for 5 days. Medium was changed at 1- to 3-day intervals and 24 h before initiating experiments.

2.3. Experimental treatment of cartilage disks

DMEM for experiments was supplemented with 10 μg/ml bovine serum albumin (BSA) to prevent nonspecific absorption of low abundance proteins. Cartilage disks were distributed as individual replicates in 96-well plates. Disks were preincubated with PKR inhibitor for 2 h and then incubated with or without human rIL-1α (10 ng/ml) for the indicated time. Disks and media were separated and stored at −70 °C for subsequent analyses.

2.4. Proteoglycan release

Cartilage proteoglycan degradation was assessed by measuring sulfated glycosaminoglycan (GAG) released into culture media using dimethyl methylene blue with chondroitin sulfate as a standard [17]. Results are expressed as μg of GAG released per disk.

2.5. Prostaglandin E_2 release

Aliquots of culture media from cartilage disks were diluted at least 1:5 and analyzed for prostaglandin E_2 (PGE_2) using Parameter Competitive ELISA Kits purchased from R&D Systems, Minneapolis, MN. In samples of media where PGE_2 was below the limit of detection even at 1:5 dilution, values are recorded as zero. PGE_2 release is reported as ρg/disk.

2.6. Cytotoxicity assays

The CytoTox-One assay (Promega Corp. Madison, WI) was used to measure lactate dehydrogenase (LDH) released into culture medium from non-viable cells in the cartilage disks as previously described [18].

2.7. Preparation of cartilage disk extracts and western blot analysis

Cellular proteins were extracted from disks using 50 μl per disk of detergent-containing buffer (20 mM Tris–HCl pH 7.8,

0.15 M NaCl, 5 mM EDTA, 1% Triton X-100, 0.1% SDS, 0.5% sodium deoxycholate, 1 mM dithiothreitol (DTT), supplemented with protease inhibitor and phosphatase inhibitor cocktails) by gentle agitation at 4 °C for 4 h. Proteins in aliquots of disk extract equivalent to one half of one disk were resolved on SDS polyacrylamide gels and transferred to nitrocellulose membranes. Transferred proteins were stained with Ponceau S to monitor protein loading and transfer. Proteins of interest were detected using specific primary antibodies and horseradish peroxidase-conjugated secondary antibodies with chemiluminescent detection. Blots were scanned and analyzed for net signal intensity using Kodak Image Station 440 software. Signals for phosphorylated eIF2α were normalized to signals obtained using antibody to β-actin or an antibody that recognizes eIF2α regardless of its phosphorylation status on parallel immunoblots. Membranes examined for specifically phosphorylated PKR were reprobed using an antibody to β-actin, and signals for phosphorylated PKR were normalized to the β-actin signals.

2.8. Statistical analysis

Statistical analysis was carried out using GraphPad Prism software. One-way analysis of variance (ANOVA) with Tukey post hoc comparison of groups was used to test for significant effects of the PKR inhibitor on IL-1α-stimulated effects in cartilage. IC50 was estimated by linear regression of GAG release (eight individual replicates for each condition) versus log concentration with standard slope and without weighting.

3. Results

3.1. Inhibition of IL-1α-stimulated effects in cartilage by a potent PKR inhibitor

PKR has been reported to play a role in cartilage degradation activated by TNFα or C2-ceramide [14]. However, PKR involvement in the catabolic effects of IL-1α was not addressed, and 2-aminopurine, the PKR inhibitor that supported this contention, is known to inhibit other processes at the doses used. To further investigate the issue, a potent cell permeable inhibitor of PKR kinase activity was tested for its effect on the degradation of proteoglycan in IL-1α-stimulated bovine articular cartilage. The measurement of GAG released into culture medium is a well-characterized assay for catabolism of proteoglycan in the cartilage extracellular matrix. IL-1α-stimulated GAG release was inhibited in dose-dependent manner by the PKR inhibitor ($P < 0.001$, estimated IC50 of 267 ηM), with complete inhibition at 1 μM ($P > 0.05$ relative to basal GAG release) (Fig. 1). IL-1-stimulated proteoglycan degradation is a metabolic process that requires live chondrocytes. There was no significant effect of the PKR inhibitor on the release of LDH at concentrations of PKR inhibitor up to 6 μM, and therefore, the inhibition of GAG release by the PKR inhibitor was not due to toxicity.

IL-1 stimulation of cartilage leads to the production of proinflammatory small molecule mediators such as PGE_2 and nitric oxide that are generated through the action of the inducible enzymes cyclooxygenase-2 (COX-2) and nitric oxide synthase II (NOS II). In order to investigate whether PKR is required for IL-1 activation of the eicosanoid pathway, the effect of the PKR inhibitor on COX-2 levels in IL-1α-activated cartilage was examined by Western blot analysis of disk extracts. COX-2 was not detected in protein extracts prepared from disks of cartilage incubated in the absence of IL-1α. IL-1α stimulation for 24 h resulted in the accumulation of COX-2 in the disks, and the PKR inhibitor reduced IL-1α-induced COX-2 accumulation in a dose-dependent manner with suppression evident at the lowest dose tested (100 ηM) and almost complete inhibition at 500 ηM (Fig. 2). PGE_2 is the principal

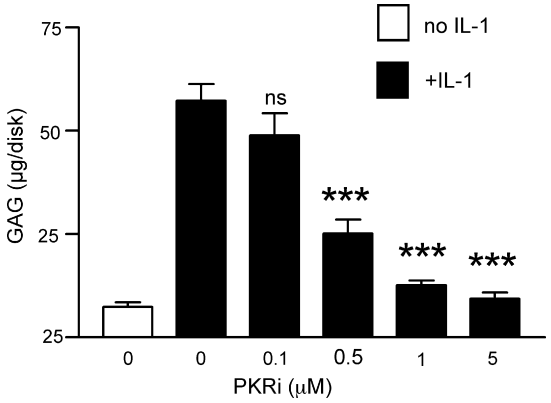

Fig. 1 – The PKR inhibitor suppresses IL-1α-stimulated cartilage proteoglycan degradation. Cartilage disks ($n = 8$) were placed individually in 200 μl DMEM supplemented with 10 μg/ml BSA in the wells of a 96-well plate. Disks were preincubated for 2 h with the PKR inhibitor (PKRi) at the indicated concentration. IL-1α (10 ηg/ml) was added (black bars) or not (open bar), and incubation was continued for 24 h. Proteoglycan degradation was assessed by assaying aliquots of culture media for GAG. Values are reported as mean + S.E.M. Significance of difference of IL-1α-stimulated GAG release in PKR inhibitor-treated disks relative to non-inhibited disks: *** $P < 0.001$; ns, not significant.

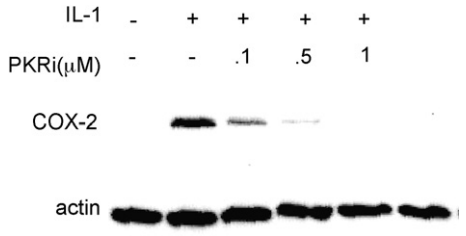

Fig. 2 – The PKR inhibitor reduces the accumulation of COX-2 in IL-1α-activated cartilage. Cartilage disks ($n = 8$) were preincubated for 2 h with the indicated concentration of the PKR inhibitor in DMEM supplemented with BSA. IL-1α was added to selected wells, and incubation was continued for 24 h. Protein extracts were prepared from cartilage disks for analysis by Western blotting using antibody to COX-2 with anti-β-actin to monitor loading and transfer. COX-2 accumulation was dependent on IL-1α and inhibited by the PKR inhibitor.

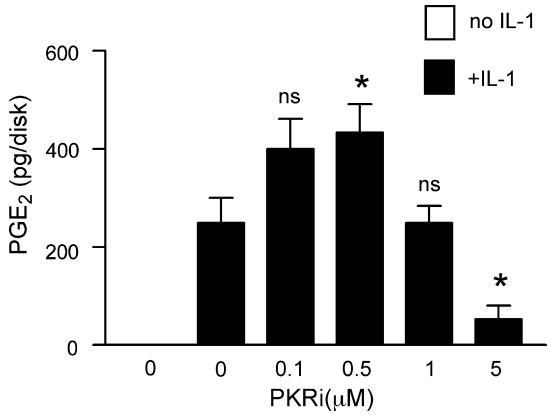

Fig. 3 – The PKR inhibitor has biphasic dose-dependent effects on IL-1α-stimulated PGE_2 production. Cartilage disks ($n = 8$) were preincubated for 2 h with the PKR inhibitor at the indicated concentration. IL-1α (10 ηg/ml) was added (black bars) or not (open bar not visible), and incubation was continued for 24 h. Harvested culture media were assayed for PGE_2 by ELISA. Significance of difference of IL-1α-stimulated PGE_2 release in PKR inhibitor-treated disks relative to non-inhibited disks: $^{*}P < 0.05$; ns, not significant.

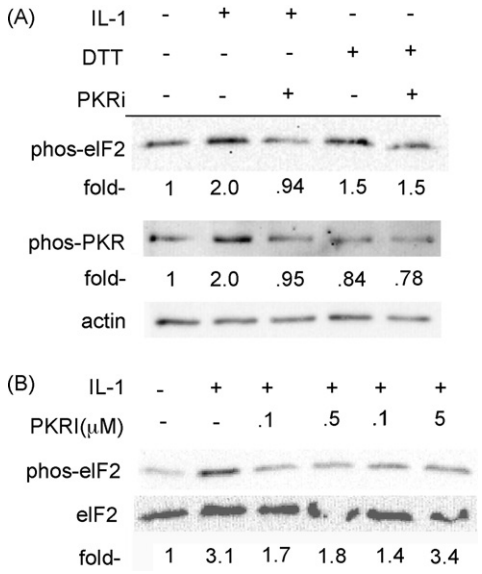

Fig. 4 – The PKR inhibitor prevents the IL-1α-stimulated increase in phosphorylation of PKR and eIF2α in cartilage. (A) Cartilage disks were preincubated for 2 h with or without 20 ηM PKR inhibitor. IL-1α (10 ηg/ml) or 3 mM DTT was added as indicated, and the incubation was continued for 30 min. Protein extracts of the disks were analyzed by Western blot for phosphorylated eIF2α, phosphorylated PKR, and β-actin. Signals for phosphorylated proteins were normalized to signals obtained using antibody to β-actin. Fold-stimulation shown below the Western blot for each phosphorylated protein is calculated as the ratio of each normalized signal relative to the normalized signal for the extract from unstimulated cartilage disks shown in lane 1. (B) Disks were preincubated for 2 h with the indicated concentrations of PKR inhibitor before the addition of IL-1α to selected wells. The incubation was continued for 24 h. Phosphorylated and total eIF2α in disk extracts were analyzed by Western blot, and the signals for the phosphorylated form were normalized to signals for total eIF2α. Fold-stimulation is determined as in the legend for Fig. 4B. IL-1α caused a rapid and prolonged increase in eIF2α phosphorylation that was inhibited in disks treated with the PKR inhibitor.

COX-2-dependent prostanoid mediator in chondrocytes, and the PKR inhibitor at 5 μM significantly suppressed IL-1α-stimulated PGE_2 production. Surprisingly, concentrations of the PKR inhibitor in the nanomolar range increased IL-1α-stimulated PGE_2 production with a significant ($P < 0.05$) effect at 500 ηM (Fig. 3).

3.2. *Phosphorylation of eIF2α in IL-1α-activated cartilage is sensitive to the PKR inhibitor*

Previous studies showed an increase in the specific phosphorylation of PKR in bovine articular cartilage detectable 6 h after co-stimulation with TNFα and IL-1α, and phosphorylated eIF2α was evident by immunocytochemical staining of chondrocytes 3 h after TNFα stimulation. In order to assess whether IL-1α alone activates PKR and leads to the phosphorylation of eIF2α, cartilage disks were preincubated for 2 h with the PKR inhibitor and then stimulated with IL-1α for various time intervals. DTT (3 mM) was used as a positive control for eIF2α phosphorylation because it reportedly activates PKR and PERK in other cell types [15,19]. Despite the presence of insulin to counter the stresses of serum-free culture [20,21] and consistent with previous reports, low level phosphorylation of PKR and eIF2α was apparent under basal conditions. IL-1α treatment increased PKR and eIF2α phosphorylation 2-fold within 30 min (Fig. 4A). Importantly, the PKR inhibitor at concentrations as low as 20 ηM almost totally prevented the change in phosphorylation of PKR and eIF2α in IL-1α-activated cartilage. DTT treatment of cartilage disks for 30 min modestly increased the phosphorylation of eIF2α but not PKR. Furthermore, eIF2α phosphorylation was not sensitive to the PKR inhibitor in this system. Hyperphosphorylated eIF2α was still evident in cartilage that had been incubated with IL-1α for 24 h (Fig. 4B).

4. Discussion

The diverse effects of IL-1 in cartilage are elicited through pathways that remain poorly defined but may involve PKR. The PKR inhibitor that was identified by Jammi and colleagues [22] inhibits IL-1α-stimulated cartilage proteoglycan degradation and suppresses the production of COX-2 in IL-1α-activated bovine articular cartilage. The potency of this inhibitor against these IL-1α-stimulated effects in cartilage explant assays is at least three orders of magnitude greater than for 2-aminopurine and is comparable to inhibition of PKR autophosphorylation in the cell-free system [22]. Low level basal phosphorylation of both PKR and eIF2α was evident in unstimulated bovine articular cartilage explants, in agreement with previous reports [12]. IL-1α treatment increased the

phosphorylation of both PKR and eIF2α in cartilage. PKR inhibitor concentrations as low as 20 ηM blocked the changes in phosphorylation of PKR and eIF2α, defining PKR as the IL-1α-activated eIF2α kinase.

These data support and extend the work of Gilbert and colleagues, providing strong evidence that the kinase activity of PKR is required for at least some of the effects of IL-1α in cartilage. The confirmation is important because the inhibition by 2-aminopurine, which is known to affect other processes and kinases including MAPKs [15,16], provided the primary support for a PKR role in cytokine-activated cartilage catabolism. The discovery of a potent selective inhibitor for PKR in a library of ATP-binding site directed compounds have provided a powerful new tool for dissecting the role of PKR in IL-1-activated cartilage. Compound #16 in the library partially restored translation inhibited by a PKR kinase domain construct in a rabbit reticulocyte cell-free translation system and inhibited poly[rI: rC]-activated PKR autophosphorylation (IC50 approximately 200 ηM) [22]. By comparison, the IC50 for 2-aminopurine was 10 mM.

The PKR inhibitor has previously been shown to be effective in cultured cells at approximately the concentrations that blocked double stranded RNA-activated PKR autophosphorylation and rescued PKR-inhibited translation. It protected against tunicamycin-induced apoptosis, prevented PKR autophosphorylation, and reduced caspase-3 activation in cultured neuroblastoma cells [23,24]. In bronchial epithelial cells, either rhinovirus infection or stimulation with a double stranded RNA analogue activated cytokine production, and this potent PKR inhibitor significantly blocked these effects at 20 ηM [25]. IL-1 has been implicated in a variety of pathological conditions, including cancer, inflammation, degenerative diseases and cardiovascular diseases [26–30]. Therefore, it is important to determine whether PKR kinase activity is involved in IL-1-activated effects in other cell types.

Although the PKR inhibitor has been shown to be selective and potent, additional studies with purified molecules are necessary to assure its specificity. IL-1α activates catabolic, anti-anabolic and proinflammatory pathways in cartilage, and we have investigated whether PKR is required in a limited subset of these effects. PKR is likely to be involved in other IL-1α-activated pathways that would, therefore, be susceptible to similar modulation by the PKR inhibitor. Certainly this should be addressed. For reasons that are unclear, the PKR inhibitor affected PGE_2 production with a biphasic dose response relationship; nanomolar PKR inhibitor concentrations that suppressed COX-2 accumulation paradoxically stimulated PGE_2 production, while low micromolar concentrations were inhibitory.

The complex regulation of the eicosanoid metabolic pathway and the possibility for diverting among alternative eicosanoids complicate identification of the mechanisms involved in the approximately 2-fold-stimulation of PGE_2 release by nanomolar concentrations of PKR inhibitor. Cyclooxygenases convert arachidonic acid liberated from cell membranes by phospholipase A2 to PGG_2 and PGH_2. Further conversion to various eicosanoid mediators requires the action of specific synthases; for PGE_2 formation, this step is catalyzed by a prostaglandin E synthase (PGES). Low dose PKR inhibitor may modulate the levels or activities of enzymes in this pathway, leading to increased PGE_2 despite the reduction in COX-2. We considered the possibility of an inhibitor-activated increase in the constitutive cyclooxygenase COX-1, but preliminary Western blot analysis did not support this mechanism (data not shown). The concept of cross talk between COX-2 and NOS II pathways remains controversial, but nitric oxide, reactive oxygen species, and derivatives have been shown to either activate or inhibit cyclooxygenase catalytic activity and PGE_2 formation [31–36]. This suggests a possible NOS II-mediated mechanism whereby nanomolar PKR inhibitor concentrations could increase enzymatic activity and PGE_2 formation. Additional studies are clearly warranted.

The early and prolonged increase in PKR inhibitor-sensitive hyperphosphorylation of eIF2α reported here, together with the prolonged activation of PKR after co-stimulation with IL-1α and TNFα [14], suggest that PKR activity could play roles in initial signal transduction as well as the delayed effects of IL-1α. The relevant target(s) of PKR kinase activity in the suppression of IL-1α-stimulated effects in cartilage may be eIF2α or some other substrate, and the molecular pathways remain to be clarified.

The data presented here demonstrate that this potent PKR inhibitor counteracts IL-1α-activated catabolic and proinflammatory effects in cartilage. IL-1 is probably the most important cytokine in the pathological destruction of cartilage in osteoarthritis, and perhaps in rheumatoid arthritis as well [3]. It increases the production and activity of degradative enzymes that damage the extracellular matrix; perhaps more importantly, it potently disrupts tissue repair by inhibiting the synthesis of the cartilage specific macromolecules, type II collagen and aggrecan, that are essential for biomechanical function. Therefore, it is critical to understand the molecular pathways that are activated by IL-1 in chondrocytes. If PKR is found to be an essential element for IL-1-stimulated effects in human articular cartilage, it should be viewed as a potential target for pharmaceutical invention in osteoarthritis. There are currently no therapeutic agents with proven disease modifying properties for this common age-related joint disease.

Acknowledgements

This work was supported in part by the National Institute of Aging (AG20987) and The Wechsler Fund.

REFERENCES

[1] Buckwalter JA, Martin J, Mankin HJ. Synovial joint degeneration and the syndrome of osteoarthritis. Instr Course Lect 2000;49:481–9.
[2] Chevalier X, Giraudeau B, Conrozier T, Marliere J, Kiefer P, Goupille P. Safety study of intraarticular injection of interleukin 1 receptor antagonist in patients with painful knee osteoarthritis: a multicenter study. J Rheumatol 2005;32:1317–23.
[3] Dayer JM. The pivotal role of interleukin-1 in the clinical manifestations of rheumatoid arthritis. Rheumatology (Oxford) 2003;42 Suppl.(2):ii3–10.

[4] Hwang SG, Yu SS, Poo H. Chun JS c-Jun/activator protein-1 mediates interleukin-1beta-induced dedifferentiation but not cyclooxygenase-2 expression in articular chondrocytes. J Biol Chem 2005;280:29780–7.
[5] Liacini A, Sylvester J, Li WQ, Zafarullah M. Inhibition of interleukin-1-stimulated MAP kinases, activating protein-1 (AP-1) and nuclear factor kappa B (NF-kappa B) transcription factors down-regulates matrix metalloproteinase gene expression in articular chondrocytes. Matrix Biol 2002;21:251–62.
[6] Williams BR. PKR; a sentinel kinase for cellular stress. Oncogene 1999;18:6112–20.
[7] Garcia MA, Gil J, Ventoso I, Guerra S, Domingo E, Rivas C, et al. Impact of protein kinase PKR in cell biology: from antiviral to antiproliferative action. Microbiol Mol Biol Rev 2006;70:1032–60.
[8] Donze O, Deng J, Curran J, Sladek R, Picard D, Sonenberg N. The protein kinase PKR: a molecular clock that sequentially activates survival and death programs. EMBO J 2004;23: 564–71.
[9] Patel RC, Sen GC. PACT, a protein activator of the interferon-induced protein kinase, PKR. EMBO J 1998;17:4379–90.
[10] Ladiges W, Morton J, Blakely C, Gale M. Tissue specific expression of PKR protein kinase in aging B6D2F1 mice. Mech Ageing Dev 2000;114:123–32.
[11] Gilbert SJ, Duance VC, Mason DJ. Identification of changes in chondrocyte gene expression in an in vivo animal model of osteoarthritis. Osteoarthritis Cartilage 2000;8:S33.
[12] Gilbert SJ, Duance VC, Mason DJ. Tumour necrosis factor alpha up-regulates protein kinase R (PKR)-activating protein (PACT) and increases phosphorylation of PKR and eukaryotic initiation factor 2-alpha in articular chondrocytes. Biochem Soc Trans 2002;30:886–9.
[13] Oliver BL, Cronin CG, Zhang-Benoit Y, Goldring MB, Tanzer ML. Divergent stress responses to IL-1beta, nitric oxide, and tunicamycin by chondrocytes. J Cell Physiol 2005;204:45–50.
[14] Gilbert SJ, Duance VC, Mason DJ. Does protein kinase R mediate TNF-alpha- and ceramide-induced increases in expression and activation of matrix metalloproteinases in articular cartilage by a novel mechanism? Arthritis Res Ther 2004;6:R46–55.
[15] Hosoi T, Matsunami N, Nagahama T, Okuma Y, Ozawa K, Takizawa T, et al. 2-Aminopurine inhibits leptin receptor signal transduction. Eur J Pharmacol 2006;553:61–6.
[16] Posti K, Leinonen S, Tetri S, Kottari S, Viitala P, Pelkonen O, et al. Modulation of murine phenobarbital-inducible CYP2A5, CYP2B10 and CYP1A enzymes by inhibitors of protein kinases and phosphatases. Eur J Biochem 1999;264:19–26.
[17] Farndale RW, Buttle DJ, Barrett AJ. Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. Biochim Biophys Acta 1986;883:173–7.
[18] Le D, Hofbauer MA, Towle CA. Differential effects of hyperosmotic challenge on interleukin-1-activated pathways in bovine articular cartilage. Arch Biochem Biophys 2006;445:1–8.
[19] Bertolotti A, Zhang Y, Hendershot LM, Harding HP, Ron D. Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. Nat Cell Biol 2000;2: 326–32.
[20] Patel CV, Handy I, Goldsmith T, Patel RC. PACT, a stress-modulated cellular activator of interferon-induced double-stranded RNA-activated protein kinase, PKR. J Biol Chem 2000;275:37993–8.
[21] Towle CA, Mankin HJ, Avruch J, Treadwell BV. Insulin promoted decrease in the phosphorylation of protein synthesis initiation factor eIF-2. Biochem Biophys Res Commun 1984;121:134–40.
[22] Jammi NV, Whitby LR, Beal PA. Small molecule inhibitors of the RNA-dependent protein kinase. Biochem Biophys Res Commun 2003;308:50–7.
[23] Page G, Rioux Bilan A, Ingrand S, Lafay-Chebassier C, Pain S, Perault Pochat MC, et al. Activated double-stranded RNA-dependent protein kinase and neuronal death in models of Alzheimer's disease. Neuroscience 2006;139:1343–54.
[24] Shimazawa M, Hara H. Inhibitor of double stranded RNA-dependent protein kinase protects against cell damage induced by ER stress. Neurosci Lett 2006;409:192–5.
[25] Edwards MR, Hewson CA, Laza-Stanca V, Lau HT, Mukaida N, Hershenson MB, et al. Protein kinase R, IkappaB kinase-beta and NF-kappaB are required for human rhinovirus induced pro-inflammatory cytokine production in bronchial epithelial cells. Mol Immunol 2007;44:1587–97.
[26] Kornman KS. Interleukin 1 genetics, inflammatory mechanisms, and nutrigenetic opportunities to modulate diseases of aging. Am J Clin Nutr 2006;83:S475–83.
[27] Apte RN, Krelin Y, Song X, Dotan S, Recih E, Elkabets M, et al. Effects of micro-environment- and malignant cell-derived interleukin-1 in carcinogenesis, tumour invasiveness and tumour-host interactions. Eur J Cancer 2006;42:751–9.
[28] Apte RN, Dotan S, Elkabets M, White MR, Reich E, Carmi Y, et al. The involvement of IL-1 in tumorigenesis, tumor invasiveness, metastasis and tumor-host interactions. Cancer Metastasis Rev 2006;25:387–408.
[29] Merhi-Soussi F, Kwak BR, Magne D, Chadjichristos C, Berti M, Pelli G, et al. Interleukin-1 plays a major role in vascular inflammation and atherosclerosis in male apolipoprotein E-knockout mice. Cardiovasc Res 2005;66:583–93.
[30] Gosselin D, Rivest S. Role of IL-1 and TNF in the brain: twenty years of progress on a Dr. Jekyll/Mr. Hyde duality of the innate immune system. Brain Behav Immun 2007;21:281–9.
[31] Mathy-Hartert M, Deby-Dupont GP, Reginster JY, Ayache N, Pujol JP, Henrotin YE. Regulation by reactive oxygen species of interleukin-1beta, nitric oxide and prostaglandin E(2) production by human chondrocytes. Osteoarthritis Cartilage 2002;10:547–55.
[32] Cuzzocrea S, Salvemini D. Molecular mechanisms involved in the reciprocal regulation of cyclooxygenase and nitric oxide synthase enzymes. Kidney Int 2007;71:290–7.
[33] Clancy R, Varenika B, Huang W, Ballou L, Attur M, Amin AR, et al. Nitric oxide synthase/COX cross-talk: nitric oxide activates COX-1 but inhibits COX-2-derived prostaglandin production. J Immunol 2000;165:1582–7.
[34] Mollace V, Muscoli C, Masini E, Cuzzocrea S, Salvemini D. Modulation of prostaglandin biosynthesis by nitric oxide and nitric oxide donors. Pharmacol Rev 2005;57:217–52.
[35] Simmons DL, Botting RM, Hla T. Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition. Pharmacol Rev 2004;56:387–437.
[36] Trostchansky A, O'Donnell VB, Goodwin DC, Landino LM, Marnett LJ, Radi R, et al. Interactions between nitric oxide and peroxynitrite during prostaglandin endoperoxide H synthase-1 catalysis: a free radical mechanism of inactivation. Free Radic Biol Med 2007;42:1029–38.