J. Pharm. Pharmacol. 1996, 48: 694–698
Received May 25, 1995
Accepted March 5, 1996

Immunological Detection of Type II Collagen Degradation: Use in the Evaluation of Anti-arthritic Therapies

C. O. CHICHESTER, H.-J. BARRACH*, G. R. SRINIVAS AND P. MITCHELL†

*Department of Pharmacology and Toxicology, University of Rhode Island, Kingston, RI 02881, *Department of Orthopaedics, Rhode Island Hospital, Providence, RI 02903, and †Pfizer Central Research, Groton, CT 06340, USA*

Abstract

Propagated Swarm rat chondrosarcoma cells, rabbit chrondrocytes (from articular cartilage of knee, shoulder and hip joints), and bovine nasal cartilage explant cultures were studied. Type II collagen (CII) and its peptide fragments were quantitated in cell medium and cell layer separately, using two previously developed assays; one assay employed a monoclonal antibody, C4F6, that reacts specifically with triple helical CII and the other assay used an antibody, E1E5, that reacts specifically with a peptide of CII.

A time-dependent increase in the content of CII and CII-derived peptides was observed in both rat and rabbit cultures. In both culture systems the majority of the native type II collagen is found associated with the cell layer (97% in rat cultures and 73% in rabbit cultures), while the major part of the CII peptides is found in the media (73% in rat cultures, 88% in the rabbit cultures). The concentration of peptides in the media reaches approximately 2 $\mu g\ mL^{-1}$ in both chondrocyte monolayer cultures after 4 days.

The CII peptide assay employing E1E5 was well suited to quantitate articular cartilage collagen degradation in explant culture. Thus it can be used to evaluate potential therapeutic agents that can modify or inhibit cartilage degradation. The assay has the added potential that it could be used in-vivo to evaluate the effectiveness of potential metalloproteinase inhibitors in animal models of osteoarthritis or in clinical trials.

In osteoarthritis the two major structural components of cartilage, collagen and proteoglycan, are lost (Hamerman 1989). Proteases that degrade articular cartilage matrix include the lysosomal proteases such as cathepsin B which are active at acidic pH, and metallo and serine proteases which are active at neutral pH. It is thought that the metalloproteases are primarily involved in matrix degradation while the serine proteases such as plasmin (Pelletier et al 1990) and lysosomal proteases (Buttle et al 1993) are involved in the activation of the metalloproteases. Numerous studies have identified increased activity or expression of metalloprotease activity in osteoarthritic cartilage including collagenase (Ehrlich et al 1978), stromelysin (Sapolsky et al 1976; Martel-Pelletier & Pelletier 1984), and the 72- and 92 kDa gelatinases (Mohtai et al 1993). Hollander et al (1994), using an immunoassay to type II collagen, have shown that they can identify and quantitate damaged collagen in osteoarthritic cartilage extracts. There has been an effort to develop specific inhibitors of the metalloproteases for use in osteoarthritis (Henderson & Davies 1991; Vincenti et al 1994). Many of these compounds are collagen synthetic peptide analogues which appear to inhibit both collagen and proteoglycan degradation in in-vitro assays, although they appear to be much more potent at inhibiting collagen degradation (Nixon et al 1991; Andrews et al 1992; Seed et al 1993). Considerable effort has gone into the development of proteoglycan markers for monitoring cartilage breakdown (Lohmander et al 1993); however, methods are also needed to evaluate the ability of these compounds to prevent the degradation of cartilage collagen in-vivo and in-vitro. We have recently developed methods to quantitate both intact triple helical type II collagen, which is the most abundant collagen in cartilage, and its peptide fragments (Srinivas et al 1993). Two separate immunoassays employing specific monoclonal antibodies were developed; one assay employs a monoclonal antibody, C4F6, that reacts specifically with triple helical collagen type II (CII) and the other assay uses an antibody, E1E5, that reacts specifically with a peptide of CII. We recently compared the effects of a series of anti-arthritic compounds on the synthesis of type II collagen in rat chondrosarcoma cultures using the assay employing C4F6 (Srinivas et al 1994). In the present study the two assays were utilized to compare both the synthesis of type II collagen and its degradation in both monolayer chondrocyte cultures as well as in explant cultures.

Correspondence: C. O. Chichester, Department of Pharmacology and Toxicology, University of Rhode Island, Kingston, RI 02881, USA.

Materials and Methods

Preparation of rat chondrosarcoma and rabbit articular chondrocytes

Chondrocytes were isolated from the propagated Swarm rat chondrosarcoma (RCS) according to previously described methods (Srinivas et al 1994). The isolated and dispersed chondrocytes were then resuspended in Dulbecco's modified Eagles medium (DMEM) supplemented with 20% foetal calf serum (Hyclone), 50 $\mu g\ mL^{-1}$ β-aminoproprionitrile (BAPN), 100 $\mu g\ mL^{-1}$ L-ascorbic acid, and 50 $\mu g\ mL^{-1}$ gentamycin sulphate and plated at a density $0{\cdot}5 \times 10^6$ cells in 1 mL medium per well of a 24-well plate (Corning 25801). After a stabilization period of 16 h, the medium was replaced with 2 mL DMEM containing 10% foetal calf serum (FCS), 15 mM HEPES, pH 7·2; 10 mM *N,N*-bis(2-hydroxyethyl)-2-aminoethane sulphonic acid, pH 7·2; 10 mM *N*-tris(hydroxymethyl)methyl-2-aminoethane sulphonic acid, pH 7·2; 50 $\mu g\ mL^{-1}$ BAPN, 100 $\mu g\ mL^{-1}$ L-ascorbic acid and 50 $\mu g\ mL^{-1}$ gentamycin sulphate.

Rabbit chondrocytes were isolated from the articular cartilage of knee, shoulder and hip joints of young 1·5-kg rabbits. Tissue was minced with a scalpel, and washed and incubated with 1 mg mL^{-1} collagenase and 2·5 mg mL^{-1} trypsin in DMEM for 6 h. The cells were then rinsed and incubated with 1 mg mL^{-1} collagenase for an additional 4 h. After rinsing, the cells were filtered through a nylon mesh filter, added to T-75 flasks and grown in DMEM containing 10% FCS and gentamycin. When the flasks became confluent, approximately two weeks later, the cells were trypsinized and set into 24-well plates at a density of $0{\cdot}75 \times 10^6$ cells/well. The rabbit chondrocyte cultures were handled as for the rat chondrosarcoma cells.

After an initial stabilization period the media in both the RCS chondrocyte and rabbit articular chondrocyte cultures were changed to 10% DMEM containing the additives above. This time point was designated as time 0. Cells and media were harvested at 0, 2, and 4 days. In the dexamethasone experiment the drug was solubilized in 95% ethanol diluted into PBS and then added at a concentration of 50 $\mu L\ mL^{-1}$ in the culture medium on day 0. At the time of harvest, protease inhibitors (10 mM ethylenediamine tetraacetic acid, 1 mM phenylmethylsulphonyl fluoride) were added to cells and medium. The cell layers were extracted in 1 M NaCl, 0·05 M Tris buffer at pH 7·4 and sonicated for 10 s at 20 W. The extracts were then centrifuged and the supernatants were collected. Aliquots of these supernatants or cell medium were used for the estimation of CII and CII peptides. DNA was determined in the cell-layer extract using the Hoechst 33258 fluorescent dye according to the method of Hinegardner (1971).

Bovine nasal cartilage explant cultures

Bovine nasal septum were obtained fresh from a slaughterhouse. Adhering connective tissue was removed under aseptic conditions and uniform cartilage plugs punched out with a leather punch. The plugs were cultured in DMEM/10% serum for several days then distributed into 24-well plates (3 cartilage plugs per well). One millilitre of Neuman and Tytell serum-free media (Gibco) was added to each well along with interleukin 1 (IL-1; human recombinant IL-1 alpha; 5 ng mL^{-1}) and metalloprotease inhibitor where appropriate. The medium was removed and fresh medium containing IL-1 added every 3–4 days. The removed medium was stored frozen until analysis.

Quantitation of type II collagen and its peptide fragments

Type II collagen and its peptides were quantitated in the cell medium and cell layer separately, using specific quantitative immunoassays (Srinivas et al 1993). The assays were performed as inhibition ELISAs, where either helical CII- or CII peptide-specific monoclonal antibodies at predetermined optimal concentrations were incubated with aliquots of cell layer extracts and cell medium for 20 h at room temperature. These mixtures were transferred onto the microtitre plates precoated with either CII (2 $\mu g\ mL^{-1}$) or CII cyanogen bromide peptides (0·5 $\mu g\ mL^{-1}$). After a 30 min incubation the plates were washed with phosphate-buffered saline, 0·05% Tween 20. The free antibody bound to the microtitre plates was then detected by using goat anti-mouse IgG second antibody conjugated to horseradish peroxidase. After incubating the plates with buffered substrates, H_2O_2, and tetramethylbenzidine, the resulting optical density was measured at 490 nm. A standard curve was prepared by incubating the antibodies C4F6 or E1E5 with increasing concentrations of purified CII or CII cyanogen bromide peptides.

Immunoblotting

Media samples from 4-day chondrosarcoma or 24-day bovine nasal cultures were analysed by immunoblotting using antibody E1E5. Tissue culture supernatants were first concentrated by lyophilization and reconstituted in 0·15 M acetic acid. Because of the foetal calf serum proteins, the media from chondrosarcoma cultures were precipitated with a final concentration of 5% trichloroacetic acid. At this concentration, collagen peptides are found in the supernatant. The supernatants were then desalted by chromatography on Bio-Gel P-2 (BioRad) and lyophilized. To facilitate analysis some preparations were treated with cyanogen bromide before immunoblotting. CII peptides were separated by SDS polyacrylamide electrophoresis on 10% high resolution tricine gels in a Bio Rad mini-gel system (Schäggar & Von Jagow 1987) and subsequently transferred to nitrocellulose membranes. The membranes were first blocked with PBS-Tween, and then incubated with antibody E1E5 in PBS-Tween for 1 h. The membranes were washed three times with PBS-Tween and the bound monoclonal antibody was detected using goat anti-mouse IgG peroxidase-labelled antibody. After washing, the bands were visualized by incubating with chromophore solution containing 4-chloro-1-naphthol and 3′-3′-diamino benzidine and hydrogen peroxide as substrate for 10 min.

Results

The inhibition ELISA utilizing monoclonal antibody C4F6 can be used to quantitate intact helical type II collagen while the assay using E1E5 can be used to quantify denatured or degraded type II collagen. Examples of the specificity of the two assays are shown in Fig. 1. These assays are specific to type II collagen in either the helical or denatured nonhelical configuration or peptide form and can be employed with no cross-interference in the same culture samples.

Chondrocyte cultures have been used extensively as a model to study proteoglycan and type II collagen synthesis. To demonstrate the applicability of the CII immunoassays to study collagen degradation, cultures of RCS and rabbit articular chondrocytes were utilized. Chondrocytes were isolated from the propagated rat chondrosarcoma or rabbit articular cartilage using collagenase treatment and then placed into culture. A time-dependent increase in the content of CII and CII-derived peptides was observed in both cultures (Table 1). The distribution of CII and collagen-derived peptides in the medium and cell layer after four days was quantitated. In both culture systems the majority of the native type II collagen is found associated with the cell layer (97% in RCS cultures and 73% in rabbit cultures), while the major part of the CII peptides is found in the media (73% in RCS cultures, 88% in the rabbit cultures). The concentration of peptides in the media reaches approximately 2 $\mu g\ mL^{-1}$ in the rat and rabbit chondrocyte monolayer cultures after 4 days.

The chondrosarcoma culture system combined with the ELISA for type II collagen content can be used for screening of anti-arthritic compounds. In this system we can quantitate

Table 1. Type II collagen and type II collagen-derived peptide production by rat chondrosarcoma and rabbit chondrocyte cultures.

	CII collagen $\mu g\ (\mu g\ DNA)^{-1}$			CII peptides $\mu g\ (\mu g\ DNA)^{-1}$		
	Day 0	Day 2	Day 4	Day 0	Day 2	Day 4
Rat chondrosarcoma	1·5 ± 0·5	3·75 ± 0·5	8 ± 1	0·05 ± 0·02	0·45 ± 0·05	0·95 ± 0·1
Rabbit chondrocytes	2·5 ± 1·0	3·0 ± 1·0	9 ± 2	0·1 ± 0	0·5 ± 0·05	0·9 ± 0·1

Chondrocytes were isolated from rabbit articular cartilage or from the propagated Swarm chondrosarcoma tumour and cultured in confluent monolayers in 24-well plates in Dulbecco's modified Eagle's medium containing 10% foetal calf serum, 50 $\mu g\ mL^{-1}$ ascorbic acid and 50 $\mu g\ mL^{-1}$ β-aminoproprionitrile. Both medium and cells were harvested at days 1, 2 and 4, and protease inhibitors were added to prevent degradation. Type II collagen and type II collagen-derived peptides were then

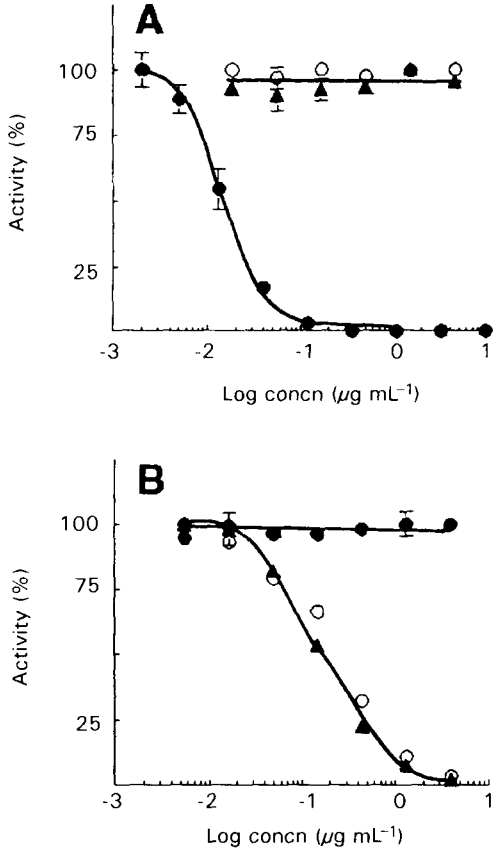

FIG. 1. Inhibition ELISA using (A) antibody C4F6 and (B) antibody E1E5. Antibody samples were incubated with increasing amounts of type II collagen (●), type II collagen-derived peptides (▲) or heat-denatured type II collagen (○). The mixtures were then transferred to microtitre plates coated with type II collagen. The binding of the antibodies to the solid phase antigen was measured by ELISA and the optical density values were read at 450 nm.

the effects of drugs that stimulate or inhibit type II collagen synthesis. Recently we tested a number of anti-arthritic compounds on collagen production in the chondrosarcoma culture system. A number of drugs including dexamethasone and diclofenac inhibited type II collagen synthesis (Srinivas et al 1994). We therefore studied the effect of inhibition of collagen synthesis on CII peptide production. At dexamethasone concentrations which inhibited the synthesis of CII (40 ng mL^{-1}) the concentration of CII peptides were also reduced (Table 2). This concentration of dexamethasone does not effect glycosaminoglycan production in this system (Srinivas et al 1994). In cell culture the amount of CII peptides appears to be dependent on the amount of newly synthesized collagen that is available for degradation.

Table 2. Effect of dexamethasone on type II collagen and type II collagen peptide production by chondrosarcoma cultures.

	Dexamethasone (ng mL^{-1})	CII collagen $\mu g\ (\mu g\ DNA)^{-1}$	CII peptides $\mu g\ (\mu g\ DNA)^{-1}$
Day 0	–	0·1 ± 0	0
Day 4	–	3·5 ± 0·25	2·2 ± 0·5
	0·4	1·4 ± 0·2	0·7 ± 0·1
	40	0·9 ± 0·1	0·5 ± 0·2

Chondrocytes were isolated from the propagated rat chondrosarcoma and cultured as previously described. After the initial stabilization period the medium was changed and medium containing 10% foetal calf serum, 50 $\mu g\ mL^{-1}$ ascorbic acid, and 50 $\mu g\ mL^{-1}$ β-aminoproprionitrile together with dexamethasone at the concentrations indicated. At the end of four days the amount of type II collagen and type II collagen-derived peptides were quantitated by ELISA. Values represent mean ± s.d. for eight replicates. $^*P < 0.01$ by one-way analysis of variance.

The chondrocytes in bovine nasal cartilage explants will degrade both the proteoglycan and collagen components of the extracellular matrix in the presence of IL-1 (Nixon et al 1991). In the case of collagen degradation, the release of collagen fragments is not observed until after 2–3 weeks of culture with IL-1. The collagen degradation is presumably due to the action of collagenase since it can be inhibited by potent collagenase inhibitors (Nixon et al 1991). Using the CII peptide assay, collagen fragments released into the media could be quantified in this assay system. In unstimulated cultures significant concentration of CII peptides could be quantitated in the media at early time points. Table 3 shows the amount of collagen fragments released from control and IL-1-treated cultures as a function of time. In other experiments significant variations in both the rate and time of maximal fragment release was observed between different cultures; however, the amount of collagen fragments released from the IL-1-treated cultures was always vastly greater than that released from the control cultures. The peptidic hydroxamic acid collagenase inhibitor (SC-44463) was able to block the IL-1-stimulated CII-peptide release from bovine nasal cartilage. Treatment with the collagenase inhibitor at a concentration of 20 nM blocked IL-1-induced collagen type II-peptide release into culture medium to well below control values (data not shown).

Immunoblotting was performed to identify the bovine nasal

Table 3. Release of type II collagen peptides ($\mu g\ mL^{-1}$) by bovine nasal cartilage explants.

	Days in culture						
	4	7	11	14	18	21	25
Control	5	12	15	10	10	5	5
IL-1	10	5	4	0	237	125	75

Explants were cultured in serum-free media in the absence and presence of 5 ng mL^{-1} IL-1. Medium was collected and fresh medium added every 3–4 days. Collagen type II peptides were quantitated in the media at the times indicated, by ELISA. Data are expressed as the concentration of peptides present at the time of media change. The numbers are corrected for the length of time that the medium was present on the cultures. Values represent mean of triplicate experiments.

organ cultures and the size of the CII peptides released into the medium of the RCS cultures using monoclonal antibody E1E5 (Fig. 2). In Western blotting E1E5 reacts with CB8, CB9.7 and cross-linked peptides which include the epitope on CB9.7 and incompletely digested peptides. In the rat chondrosarcoma culture media, E1E5 recognizes a peptide substantially greater in molecular weight than CB8 while in the bovine nasal cultures peptides it reacted with several peptides of molecular weight of 30 kDa and greater. To compare the epitopes recognized by E1E5 in culture media, the culture supernatants were treated with cyanogen bromide so that the media peptides could be directly compared with the CII cyanogen bromide standards. In rat chondrosarcoma culture supernatants, E1E5 stained one major band of the size of CB8. In control bovine nasal culture supernatants, a major band corresponding to CB8 and a minor band corresponding to CB 9.7 were seen. Western blot analysis of the IL-1-treated culture media was only possible after cyanogen bromide treatment and two bands in the region of CB8 were stained. It is possible that in these samples some of the peptides measured in ELISA were lost in sample preparation due to their small size.

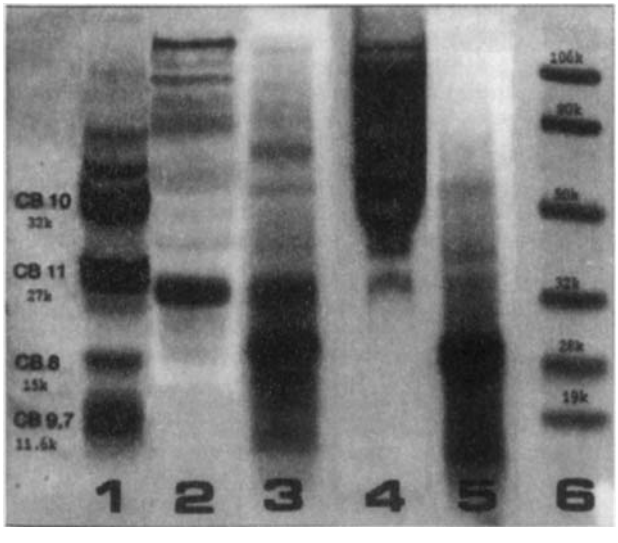

FIG. 2. Immunoblotting of collagen type II released into the media from RCS and cultured bovine nasal cartilage explants. Lane 1, cyanogen bromide fragments of type II collagen stained with Coomassie blue; lane 2, media proteins from RCS cultures immunostained with antibody E1E5; lane 3, media protein from RCS culture treated first with cyanogen bromide and then immunoblotted with antibody E1E5; lane 4, media from bovine cartilage explants immunostained with E1E5; lane 5, media from bovine cartilage explants first treated with cyanogen bromide and then immunostained.

Discussion

This study examined the degradation of CII peptides in several different culture systems. In both the rat chondrosarcoma and rabbit articular chondrocyte monolayer cultures, there was a steady increase in the amount of peptides released into the medium over a four-day period. In both cultures, at the end of four or five days in culture, the need to change the media limited the extent of peptide concentrations that could be obtained. The levels of CII and CII peptides generated in the monolayer RCS cultures after four days of culture was similar to that of the rabbit articular chondrocytes. DePasquali et al (1986) measured collagenase activity in RCS cultures and rabbit articular chondrocytes grown under similar conditions to this study. They found similar amounts of collagenase activity in both cultures. It is not surprising that the amount of CII peptides produced in both culture systems was similar since both cultures produced equivalent amounts of CII substrate and collagen-degrading enzymes.

A much greater release of peptides was seen in the bovine nasal explant cultures. In the bovine cartilage explants, culture media concentrations of up to 50 $\mu g\ mL^{-1}$ CII peptides were obtained, while in both chondrocyte cultures tested values were in the range of 1–2 $\mu g\ mL^{-1}$. Several factors may contribute to the higher CII peptide concentration in explant cultures. The cartilage explants are cultured over relatively long periods of time without changing the medium, thus allowing for a greater build-up of peptides. In addition, the explant cultures have a much greater quantity of collagen type II available for degradation. In isolating the chondrocytes for the monolayer cultures, almost all of the extracellular matrix is digested. Therefore, in the monolayer culture systems, the amount of degraded CII is dependent on the prior synthesis of substrate.

The amounts of CII peptide produced in the explant cultures were dramatically stimulated by IL-1 treatment with a maximum occuring at the third week of treatment. The production of the peptides eventually tapers off as the substrate is depleted. The release of CII peptides correlates with the appearance of active collagenase in the media (P. Mitchell unpublished data). The IL-1-induced CII-peptide release from explant cultures was inhibited by the hydroxamic acid collagenase inhibitor SC-44463. The compound is also effective in preventing peptide release in the rat chondrosarcoma cultures. Although SC-44463 has been shown to inhibit interstitial collagenase, it can inhibit other metalloproteases such as the type IV collagenases (Reich et al 1988). It is probable that the generation of peptides is dependent upon collagenase which makes the first cleavage in the molecule but also on other metalloproteases that can degrade the released peptides.

The major peptide released into the control media in the bovine nasal cartilage system has a molecular weight of approximately 27 kDa according to collagen standards. This, as well as larger peptides in culture media recognized by E1E5, could be on either the C or N terminal side of the collagenase cleavage site. In type II collagen, collagenase cleaves to the

Gly-Ile peptide bond in a sequence located between the CB peptides 8 and 9·7 (Miller et al 1976), both of which are recognized by E1E5. We have digested type II collagen with purified neutrophil collagenase and substantiated that both fragments are recognized by E1E5 (data not shown). Since collagenase cleavage fragments are 70 and 30 kDa, respectively, further protease degradation must occur. It is probable that a number of fragments of type II collagen are produced through the action of other matrix metalloproteases.

In conclusion we have shown that the CII peptide assay employing E1E5 is well suited to quantitate articular cartilage collagen degradation in explant culture. Thus it can be used to evaluate potential therapeutic agents that can modify or inhibit cartilage degradation. IL-1-treated bovine nasal explant cultures have been used for screening collagenase inhibitors. A number of metalloprotease inhibitors have been shown to selectively inhibit the breakdown of collagen in this model (Nixon et al 1991; Goldberg et al 1995). The typical end point is the release of hydroxyproline into the culture media supernatant. The CII peptide assay has an advantage over hydroxyproline analysis in that it can be performed on large numbers of samples in short periods of time without the need for prior hydrolysis. The assay has the added potential that it could be used in-vivo to evaluate the effectiveness of potential metalloproteinase inhibitors in animal models of osteoarthritis or in clinical trials.

References

Andrews, H. J., Plumpton, T. A., Harper, G. P., Cawston, T. E. (1992) A synthetic peptide metalloproteinase inhibitor, but not TIMP, prevents the breakdown of proteoglycan within articular cartilage in vitro. Agents Actions 37: 147–154

Buttle, D. J., Handey, C. J., Ilic, M. Z., Saklatuala, J., Murat, M., Barratt, A. J. (1993) Inhibition of cartilage proteoglycan release by a specific inactivator of cathepsin B and inhibitor of matrix metalloproteases: evidence for two converging pathways of chondrocyte-mediated proteoglycan degradation. Arthritis Rheum. 36: 1709–1717

DiPasquale, G., Caccise, R., Pasternak, R., Caputo, C. B., Conaty, J., Hubbs, S. (1986) Lack of response of rat chondrosarcoma chondrocytes to interleukin I to produce proteoglycan and collagen degrading enzymes. Res. Comm. Chem. Pathol. Pharmacol. 52: 333–339

Ehrlich, M. G., Houle, P. A., Vigliani, G., Mankin, H. J. (1978) Correlation between articular cartilage collagenase activity and human osteoarthritis. Arthritis Rheum. 24: 761–766

Goldberg, R. L., Spirito, S., Doughty, J. R., Ganu, V., Heinegard, D. (1995) Timed dependent release of matrix components from bovine articular cartilage after IL-1 treatment and their inhibition by matrix metalloproteinase inhibitors. Trans. Ortho. Res. Soc. 41: 125

Hamerman, D. (1989) The biology of osteoarthritis. N. Eng. J. Med. 320: 1322

Henderson, B., Davies, D. E. (1991) The design of inhibitors of cartilage breakdown in osteoarthritis: current research and prospectives for pharmacological intervention. In: Russel, R. G. G., Dieppe, P. A. (eds) Osteoarthritis London: IBC Technical Services, pp 203–215

Hinegardner, R. T. (1971) An improved fluorometric assay for DNA. Anal. Biochem. 39: 197–201

Hollander, A. P., Heaphfield, T. F., Webber, C., Iwata, Y., Bourne, R., Rorabeck, C., Poole, R. (1994) Increased damage to type II collagen in osteoarthritic articular cartilage by new immunoassay. J. Clin Invest. 93: 1722–1732

Lohmander, S. L., Hoerrner, L. A., Lark, M. W. (1993) Metalloproteinases, tissue inhibitor and proteoglycan fragments in knee synovial fluid in human osteoarthritis. Arthritis Rheum. 36: 181–189

Martel-Pelletier, J., Pelletier, J.-P. (1984) Metalloproteinases: neutral proteases capable of proteoglycan digesting activity in osteoarthritic and normal human articular cartilages. Arthritis Rheum. 27: 305–312

Miller, E. J., Harris, E. D., Chung, E., Finch, J. E., McCroskery, P. A., Butler, W. T. (1976) Cleavage of type II and III with mammalian collagenase: site of cleavage and primary structure at the NH_2 terminal portion of the smaller fragment released from both collagens. Biochemistry 15: 787–792

Mohtai, M., Smith, R. L., Schurman, D. J., Tsuji Y., Torti, F., Hutchinson, N., Stetier-Stevenson, W. G., Goldberg, G. I. (1993) Expression of 93-KD type IV collagenase/gelatinase (gelatinase B) in osteoarthritis and its induction in normal human articular cartilage by interleukin 1. J. Clin. Invest. 92: 179–185

Nixon, J., Bottomley, K. M., Broadhurst, M. S., Brown, P. A., Johnson, W. A., Lawton, G., Marley, J., Sedwick, A. D., Wilkinson, S. F. (1991) Potent collagenase inhibitors prevent interleukin-1-induced cartilage degradation in vitro. Int. J. Tissue React. 3: 237–241

Pelletier, J.-P., Mineau, F., Faore, M. P., Martel-Pelletier, J. (1990) Imbalance between the mechanisms of activation and inhibition of metalloproteases in the early lesions of experimental osteoarthritis. Arthritis Rheum. 33: 1466–1476

Reich, R., Thompson, E. W., Iwamoto, Y., Martin, G. R., Deason, J. R., Fuller, G. C., Miskin, R. (1988) Effects of inhibitors of plasminogen activator, serine proteinases, and collagenase IV on the invasion of basement membranes by metastatic cells. Cancer Res. 48: 3307

Sapolsky, A. I., Keisler, H., Howell, D. S., Woessner, J. F. (1976) Metalloproteases of human articular cartilage that digest cartilage proteoglycans at neutral and acid pH. J. Clin. Invest. 58: 1030–1041

Schägger, H., Von Jagow, G. (1987) Tricine-sodium dodecylsulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Anal. Biochem. 166: 368–379

Seed, M., Ismaiel, S., Cheuny, C. Y., Thomson, T. A., Gardner, C. R., Atkins, R. M., Elson, C. J. (1993) Inhibition of interleukin 1 beta induced rat and human cartilage degradation in vitro by the metalloproteinase inhibitor U2739. Ann. Rheum. Dis. 52: 37–43

Srinivas, G. R., Barrach, H.-J., Chichester, C. O.(1993) Quantitative immunoassays for type II collagen and its cyanogen bromide peptides. J. Immunol. Methods 159: 53

Srinivas, G. R., Chichester, C. O., Barrach, H.-J., Matoney, A. L. (1994) Effects of antiarthritic agents on the synthesis of type II collagen and glycosaminoglycans in rat chondrosarcoma cultures. Agents Actions 41: 193–199

Vincenti, M. P., Clark, I. M., Brinckerhoff, C. E. (1994) Using inhibitors of metalloproteinases to treat arthritis – easier said than done? Arthritis Rheum. 37: 1115–1126