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Effects of Meloxicam, Compared with other NSAIDs, on Cartilage Proteoglycan Metabolism, Synovial Prostaglandin E_2, and Production of Interleukins 1, 6 and 8, in Human and Porcine Explants in Organ Culture

K. D. RAINSFORD*†, C. YING† AND F. C. SMITH‡

**Division of Biomedical Sciences, School of Science and Mathematics, Sheffield Hallam University, Sheffield S1 1WB, UK, †Department of Biomedical Sciences, McMaster University Faculty of Health Sciences, Hamilton, Ontario L8N 3Z5, Canada, and ‡Sir William Osler Health Institute, 565 Sanatorium Road, Hamilton, Ontario L9C 7N4, Canada*

Abstract

Some non-steroidal anti-inflammatory drugs (NSAIDs) can accelerate joint damage in osteoarthritis by enhancing the production of pro-inflammatory cytokines or inhibiting cartilage proteoglycan synthesis. Meloxicam, a new NSAID, was compared with standard NSAIDs for its effect on proteoglycan synthesis and degradation in human and porcine cartilage explants, as well as the production of prostaglandin E_2 (PGE_2) and interleukins 1 and 6 by human synovial tissue explants in-vitro.

Meloxicam at submicromolar concentrations inhibited synovial PGE_2 production but, up to therapeutic drug concentrations (≤ 4 μM), did not affect synovial production of the pro-inflammatory cytokine IL-1. In contrast, hydrocortisone, 10 μM, a positive control, inhibited release of this cytokine, and indomethacin, 100 μM, increased its production. The lack of effects of meloxicam were evident irrespective of intrinsic IL-1 bioactivity of the synovia, production of IL-1 inhibitors or time of incubation. Production of the part anti-inflammatory cytokine IL-6, was significantly increased by therapeutic concentrations of meloxicam, as well as by indomethacin. Another major pro-inflammatory cytokine, IL-8, was unaffected by therapeutic concentrations of meloxicam. Meloxicam, 0·1–4·0 μM, did not affect cartilage proteoglycan production whereas indomethacin, 100 μM, significantly reduced synthesis of these macromolecules.

Thus meloxicam, at concentrations within the therapeutic range and at which pronounced inhibition of prostaglandin production is evident, affects neither cartilage proteoglycan production nor the production of those cytokines likely to be important in cartilage destruction.

Meloxicam is a new non-steroidal anti-inflammatory drug (NSAID) which, like other acidic enolcarboxamides, has proven highly potent (Wiseman 1985; Lombardino & Wiseman 1987; Engelhardt et al 1995; Rainsford 1996). Meloxicam has an optimum plasma $t\frac{1}{2}$ (approximately 20 h), for once-daily dosage and does not accumulate or show prolongation of plasma $t\frac{1}{2}$ in the elderly (Rainsford 1996). In standard animal models this drug has an anti-inflammatory potency slightly greater than that of piroxicam, with relatively low gastrointestinal ulcerogenicity (Engelhardt et al 1995). Meloxicam is, therefore, a major advance over other enolcarboxamides on the basis of optimizing the pharmacokinetic properties, so reducing adverse side effects and maintaining anti-inflammatory potency. Meloxicam appears to have a favourable gastrointestinal safety profile in man (Distel et al 1996), possibly because of its selective inhibition of cyclooxygenase-2 relative to cyclooxygenase-1 (Churchill et al 1996; Engelhardt et al 1996).

Recently, it has been suggested that some NSAIDs might protect against enhanced cartilage and bone destruction in osteoarthritis whereas others (e.g. indomethacin) might accelerate joint damage (Rashad et al 1989; Jones & Doherty 1992; Rainsford et al 1992), possibly as a result of inhibitory effects on cartilage proteoglycan synthesis (Jones & Doherty 1992; Rainsford et al 1992) or enhanced production of cartilage/bone-destructive interleukin-1 (IL-1) (Bonta & Elliott 1992), or both. Clearly, it is important to establish if meloxicam has any effects on the mediators associated with joint destruction or if it affects proteoglycan metabolism.

In this study the effects of meloxicam on cartilage proteoglycan metabolism, prostaglandin E_2 (PGE_2) synthesis, and on the production of the pro-inflammatory interleukin IL-1, and that of IL-6 were investigated in-vitro. As the production of IL-6 is induced by cyclooxygenase-2-produced PGE_2 (Hinson et al 1996) and IL-6 stimulates production of the tissue inhibitor of metalloproteinases-1 and certain acute phase proteins (Graeve et al 1993) it might be regarded as having partial anti-inflammatory activity (Whicher & Westacott 1992).

Methods

Cartilage proteoglycan metabolism

Sections of cartilage were obtained from patients with osteoarthritis who had undergone elective hip and knee surgery. The subjects had previously taken only paracetamol or NSAIDs, but not corticosteroids or other anti-rheumatic or immunosuppressive agents. Pig cartilage was also aseptically excized from the hip joints of normal animals (Rainsford et al 1989). These explants (approximately 10 mg wet weight) were incubated in 25-well sterile plastic plates under organ-culture

Correspondence: K. D. Rainsford, Division of Biomedical Sciences, School of Science and Mathematics, Sheffield Hallam University, Pond Street, Sheffield S1 1WB, UK.

conditions (Rainsford et al 1989) with 10 Ci $well^{-1}$ of $Na_2{}^{35}SO_4$ (Amersham UK) in 1·5 mL Dulbecco's Modification of Eagle's Medium (DMEM) containing penicillin (200 units mL^{-1}) and streptomycin (200 units mL^{-1}) with 0·1–100 μM meloxicam (in 0·1% EtOH in DMEM), 20 μM diclofenac sodium, 4 or 20 μM indomethacin or 4 μM piroxicam (in 0.1% EtOH in DMEM). Indomethacin was prepared as a sodium salt by dissolving the drug in a small amount of 1 M $NaHCO_3$ then adding DMEM. Controls were incubated with medium alone or, when ethanol was added to the drug mixture, this solvent was added to the control media. Ethanol (0.1%) has previously been found to have no effect on proteoglycan synthesis or degradation in either human or pig cartilage (Rainsford, unpublished studies). In some experiments human recombinant IL-1α (National Institute for Biomedical Standards and Control, Potters Bar, Herts), 20 units in each well, was added to promote proteoglycan turnover and thus mimic conditions in inflamed joints. The incubations were performed in an atmosphere of 5% CO_2 in air at 37°C and terminated 6 or 24 h later. The glycosaminoglycans in the media were isolated by precipitation with 2% (w/v) cetylpyridinium chloride (Weibkin & Muir 1977; Rainsford et al 1989). The radioactivity in the precipitated fraction was determined by scintillation counting and concentration of glycosaminoglycans was assayed by the dimethylmethylene blue method (Taylor's blue; Serva; Farndale et al 1982). The proteoglycans were extracted from thinly sliced sections (ca 0·01–0·05 mm) of the cartilage explants with 4 M guanidine hydrochloride in the presence of the protease inhibitors, EDTA.Na (10 μM), 6-amino-*n*-hexanoic acid (5 μM) and benzamidine hydrochloride (50 μM) as described elsewhere (Bayliss & Roughley 1985; Pottenger et al 1985). The guanidine hydrochloride extract was dialysed against phosphate-buffered saline at 4°C with the aforementioned protease inhibitors added, and then precipitated with 2% w/v cetylpyridinium chloride. The radioactivity in the precipitated and supernatant fractions was determined by liquid scintillation counting and the concentration of glycosaminoglycans by dimethylmethylene blue assay (Farndale et al 1982) with shark chondroitin sulphate A (Sigma, Poole, Dorset) used as standard. To identify the proportion of high- and low-molecular weight proteoglycans, some samples of human tissue previously incubated in the drugs and radioactive [^{35}S]sulphate were extracted with guanidine hydrochloride and chromatographed on Sepharose 2B-CL as described elsewhere (Bayliss & Roughley 1985).

Prostaglandin production by human synovial tissues

Explants of synovial tissues were obtained from osteoarthritis patients undergoing hip or knee surgery. The organ culture comprised approximately 1–2 mg wet weight of finely sectioned tissue in 1·0 mL DMEM with 5% foetal calf serum, penicillin and streptomycin (200 units mL^{-1} each) (Rainsford et al 1989) in 25-well sterile culture dishes. Particular care was taken to dissect away all adherent fat from the synovia. Histological examination confirmed the absence of fat and showed that most of these samples were extensively inflamed. Samples were selected from subjects who had not received steroids or disease-modifying anti-rheumatic drugs; usually they had received only paracetamol or NSAIDs for pain relief. The tissues were preincubated in DMEM + 5% foetal calf serum for 24 h to eliminate any residual effects of drugs. The medium was changed and the NSAIDs, prepared as described above, were added in the concentrations indicated in the Results section. After termination of incubation, the prostanoids in the media were isolated and purified on SepPak (Millipore, USA) C_{18} reverse-phase mini-columns. The methyl formate fractions thus obtained (Rainsford et al 1989) were dried and the residues, after being resuspended in recommended radioimmunoassay (RIA) buffer, were assayed for PGE_2 content by RIA (Rainsford et al 1989) using commercially available kits (Amersham, Toronto, ON, Canada).

Synovial production of interleukins

Human synovial tissue was cultured as described above. The tissues were placed in organ culture overnight in 25-well dishes with 1.5 mL of DMEM, 5% foetal calf serum, and 200 units mL^{-1} each of penicillin and streptomycin (Rainsford et al 1989, 1996) to achieve stabilization. After the organ cultures were pre-incubated, the medium was changed and the drugs added as above. The incubations were terminated after 20, 24 or 72 h and, after harvesting, the medium was dialysed at 4°C against sterile phosphate-buffered-saline to remove any endogenous prostaglandins which affect the conditions of the IL-1 bioassay.

The bioactive IL-1 concentration was determined by use of D-10.(N4).M cells, a D10.G4.1 T-helper subline (Hopkins & Humphreys 1989; Hopkins et al 1990; Rainsford et al 1996) kindly provided by Dr Stephen Hopkins (University of Manchester, Manchester, UK). Bioassays were performed after serial dilution of the media into the assay buffer. This assay is highly specific for IL-1, with no interference being evident from IL-2 or IL-6 (Hopkins & Humphreys 1989). In some experiments polyethylene glycol-8000 (PEG-8000) was added to remove endogenous inhibitors of IL-1 using methods described elsewhere (Hopkins et al 1990). A standard of human recombinant IL-1α, at concentrations of 0·4–32·5 units mL^{-1} (National Institute for Biological Standards and Control, Potter's Bar, Herts, UK) was used in each assay. In some experiments hydrocortisone was employed as a standard inhibitor for comparison with published studies (Rainsford et al 1996). The protein content of the synovial tissues, in these and in the prostaglandin studies, was determined by the method of Lowry et al (1951).

IL-1α, IL-1β and IL-6 were assayed by enzyme-linked immunoassay (ELISA) using commercial kits (Amersham Canada Inc., Toronto, Canada) according to the manufacturer's instructions.

Results

Proteoglycan synthesis

The in-vitro effects of meloxicam were studied in human articular cartilage from ten individuals and pig articular cartilage from six pigs. The effects of the drugs were determined in a range of tissue samples, especially where they were from man and had pathological variability. Where sufficient samples of human cartilage were available, these were cultured in the presence and absence of human recombinant IL-1α to mimic conditions in inflamed joints where there might be effects of this cytokine on proteoglycan turnover. In human tissues with high, but not low, proteoglycan synthetic rates, this cytokine caused a statistically-significant reduction (Student's *t*-test $P < 0.01$) in proteoglycan synthesis (Fig. 1a).

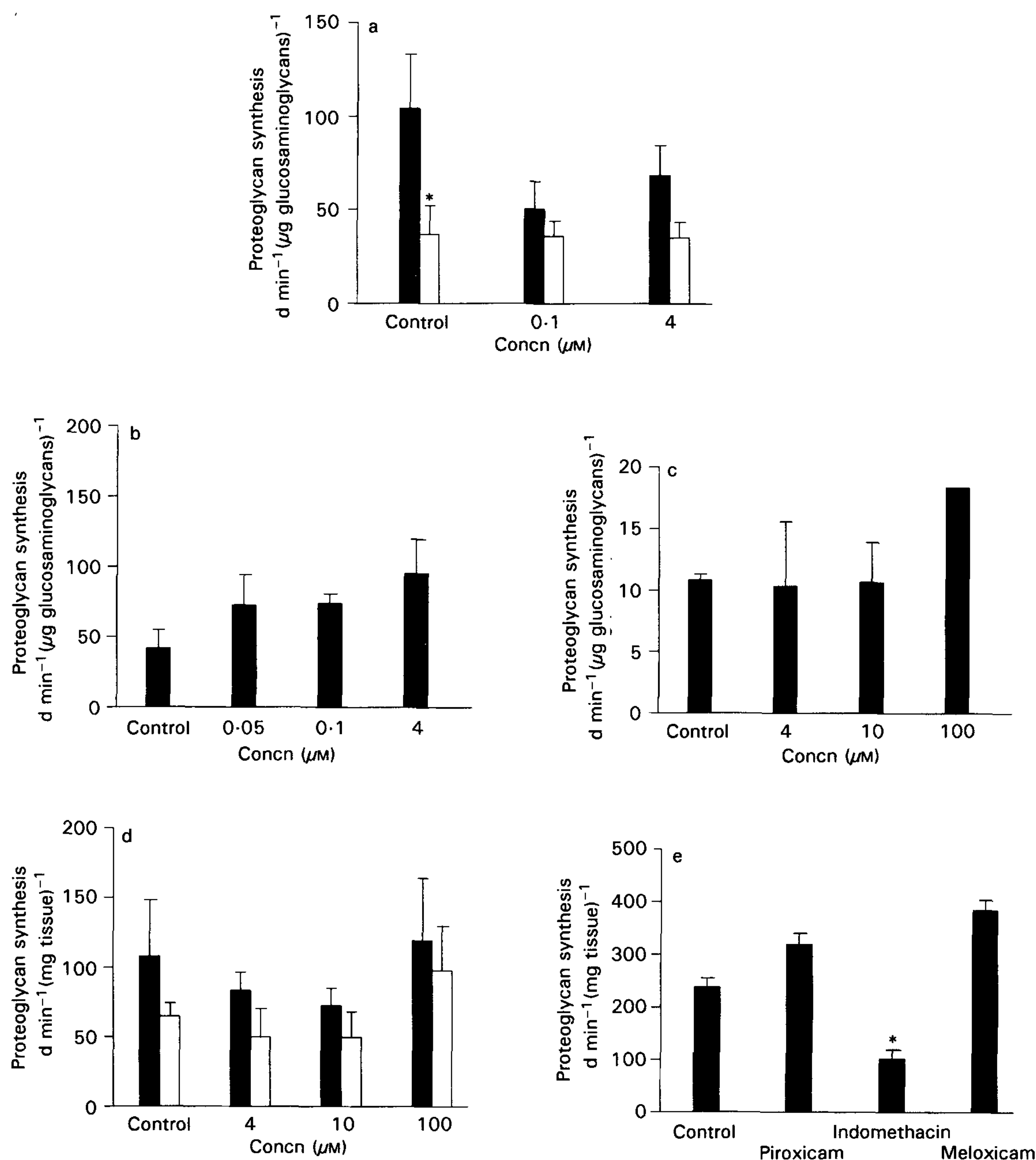

FIG. 1. Effects of meloxicam and other NSAIDs on the synthesis of radioactive sulphate-labelled proteoglycans in human articular cartilage samples from different osteoarthritis patients incubated in the presence or absence of IL-1α. The cartilage, $^{35}SO_4^{2-}$ and drug were incubated together for 24 h except for (d) for which the cartilage proteoglycans were pre-labelled with $^{35}SO_4^{2-}$ for 24 h, the cartilage was then washed free from radioactivity with 1 M Na_2SO_4 in saline, and drug, with or without IL-1α, was added for a further 24 h. This experiment shows that meloxicam at ≤ 100 μM has no effect on cartilage degradation. The effects of various NSAIDs, 20 μM each, on proteoglycan synthesis is shown in (e). Indomethacin induced a significant reduction (Student's *t*-test, $P < 0{\cdot}01$) in proteoglycan synthesis. IL-1α significantly reduced (Student's *t*-test, $P < 0{\cdot}01$) synthesis of proteoglycans in the high biosynthetic group (a). It should be noted that 4 μM drug concentration corresponds approximately to synovial concentrations achieved during therapy. All values are means ± s.e.m. (n = 5 group⁻¹).

Representative data showing the effects of the NSAIDs on proteoglycan synthesis or degradation (in pre-$^{35}SO_4^{2-}$ tissues) in human tissues are shown in Figs 1a–1e, and those from pigs in Figs 2a–2c. In all these figures it is apparent that, irrespective of drug concentration, presence or absence of IL-1α, or time of incubation, meloxicam had no inhibitory effects on proteoglycan synthesis or degradation. High concentrations (100 μM) were added to amplify effects and to obtain toxicity and safety data. Although this drug concentration of 100 μM is probably never achieved in-vivo, the results showing lack of inhibitory effects at these exceptionally high concentrations do give an indication of a safety margin with meloxicam. No significant differences were observed between meloxicam and control treatments in the radiosulphate-labelled proteoglycans in cartilage from pre-$^{35}SO_4^{2-}$ labelled tissue from man (Fig. 1d) or pigs (Fig. 2b) incubated with or without IL-1α. This suggests that meloxicam has no effect on cartilage proteoglycan degradation (Figs 1d and 2b). Of the other NSAIDs, only indomethacin inhibited proteoglycan synthesis, this being evident after 24 h incubation with 20 μM of the drug

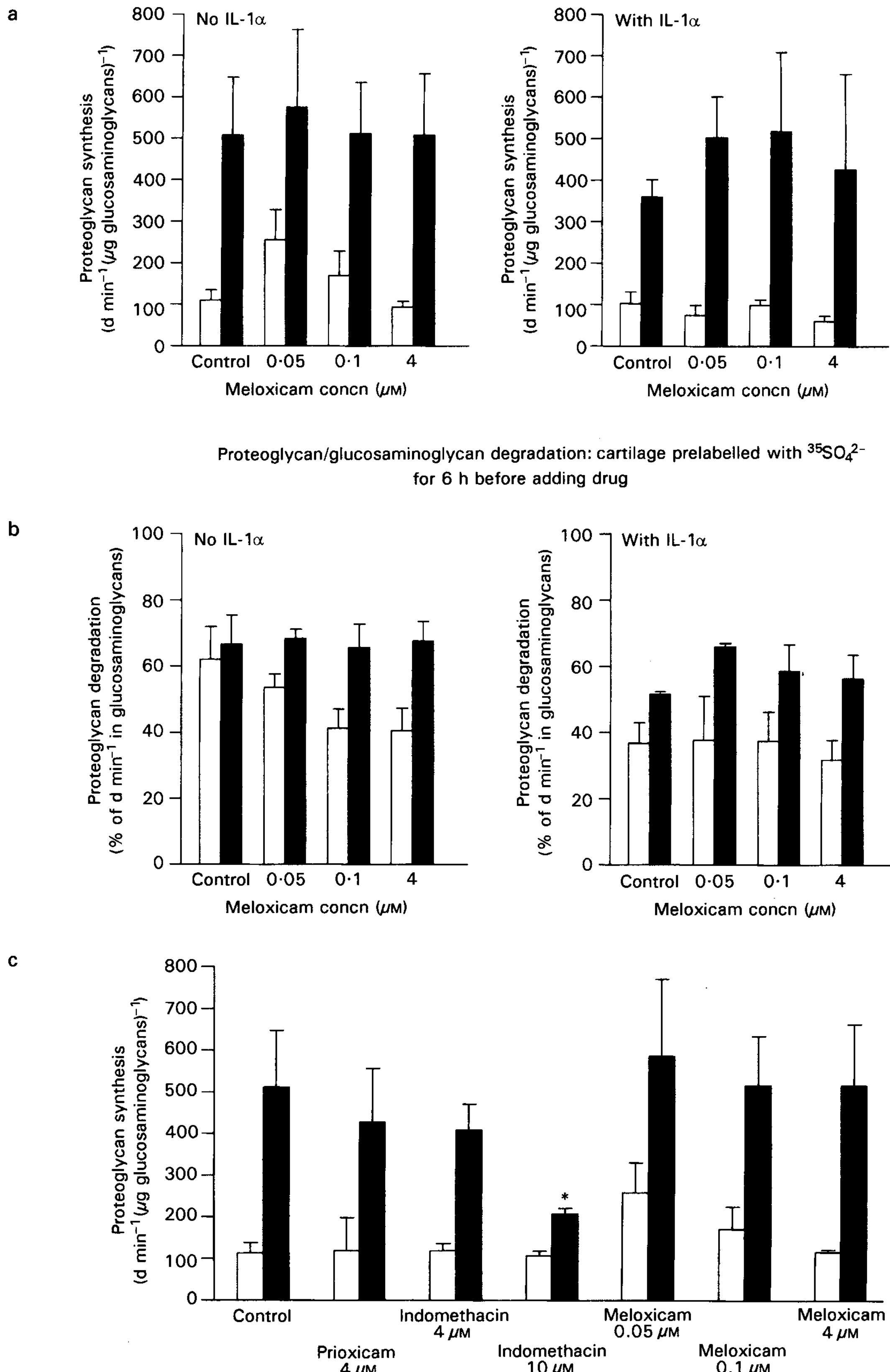

FIG. 2. The effects of meloxicam, compared with other NSAIDs, on hip articular cartilage from normal Large White × Landrace pigs. For (a) and (c) the cartilage was incubated for 6 or 24 h with drug and radioactive sulphate in the presence or absence of IL-1α. The effects of preincubation with radioactive sulphate for 6 h before addition of meloxicam, with or without IL-1α, for 24 h are shown in (b). All values are means ± s.e.m. (n = 5 group^{-1}). Indomethacin (10 μM) induced a significant reduction (*P < 0·05, Student's t-test) compared with control after 24 h incubation.

in human tissues (Fig. 1e) and at 10 μM in porcine cartilage (Fig. 2c).

Chromatography of the proteoglycan extracts on Sepharose 2B-2CL from pooled human tissue incubates (n = 10/group) with meloxicam or indomethacin is shown in Fig. 3. While there is some variability in the proportion of peaks in different fractions, it is clear that the proteoglycans synthesized from cartilage incubated with 4 μM meloxicam showed, qualitatively, a greater proportion of high-molecular weight components labelled with radiosulphate than those from cartilage treated with 4 μM indomethacin.

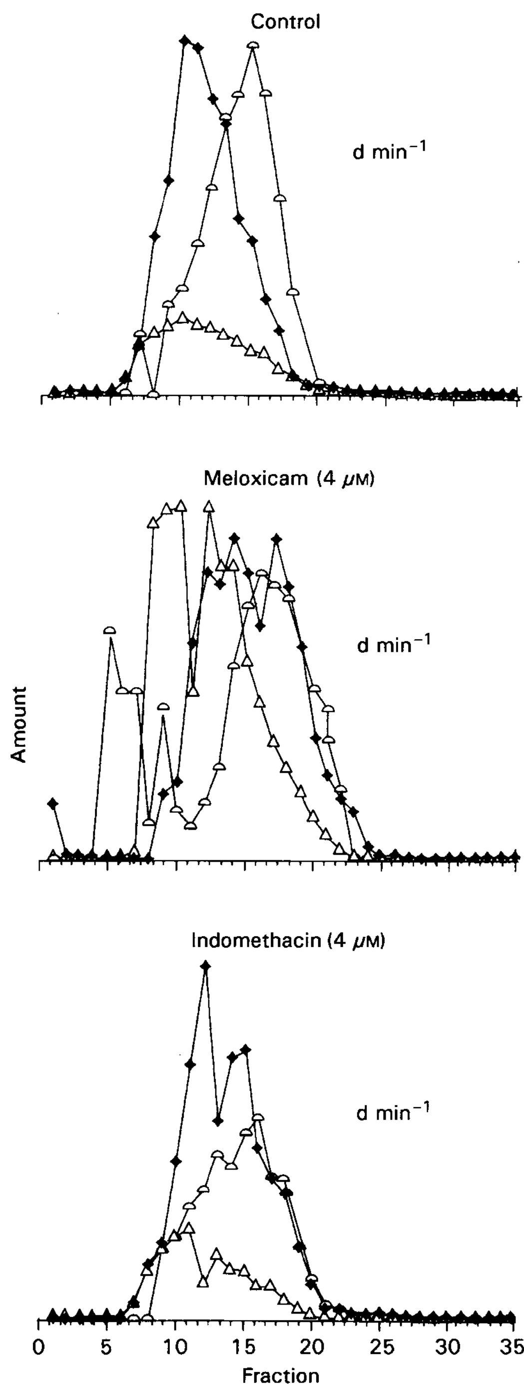

FIG. 3. Column chromatography, on Sepharose 2B-CL, of pooled (n = 10) human knee cartilage from control (a), 4 μM meloxicam (b) or 4 μM indomethacin (c) -treated tissues showing, qualitatively, an increased proportion of high-molecular weight proteoglycan components with meloxicam treatment and marked reduction with indomethacin.

Synthesis of prostaglandin and interleukins 1, 6 and 8

Synovial PGE_2 production was consistently inhibited by meloxicam at $\geq 0{\cdot}1$ μM (Table 1) and the effects of the drug were more potent than other reference NSAIDs (Fig. 4). In contrast, the production of bioactive IL-1 was unaffected up to therapeutic concentrations of meloxicam (≤ 4 μM). This was irrespective of the intrinsic IL-1 activity of the tissue, dilution of incubation media for bioassay, or the presence of inhibitors of IL-1 removable by PEG-8000 (Table 1, Figs 5a and 5b). The lack of effects of meloxicam on IL-1 production by synovia determined by bioassay were confirmed in ELISA assays for IL-1α (Table 1). Although a slight increase in IL-1 bioactivity was evident in some samples incubated with 10 μM meloxicam, again the effects were not statistically significant (Table 1; Group B; Student's *t*-test $P > 0{\cdot}05$). The effects of meloxicam on the production of IL-1 appeared to be unrelated to the inhibitory effects of this drug on PGE_2 production by synovia; the concentration of the drug required for inhibition of PGE_2 production being well below that at which negative effects on IL-1 have been observed (Table 1, Fig. 4).

In contrast with the effects of meloxicam on IL-1 production, it was found that indomethacin at 100 μM, but not 10 μM, stimulated production of this cytokine from synovia (Table 1). The effects of meloxicam and indomethacin are to be contrasted with that of the steroid, hydrocortisone 10 μM which, as expected of this positive control, inhibited production of IL-1 (Table 1).

Compared with the effects on IL-1 production, meloxicam up to therapeutic concentrations (≤ 4 μM) increased production of IL-6 by synovia (Table 1). A similar, concentration-related, increase in IL-6 production was observed with indomethacin (Table 1).

The production by human synovia of another pro-inflammatory cytokine, namely IL-8, whose actions have been linked to IL-1, were studied in two experiments and were found to be unaffected by meloxicam. In the first experiment the control mean values were 64 244 pg IL-8 mg^{-1} protein compared with 10 μM meloxicam which produced 67 656 pg IL-8 mg^{-1}. In the second experiment the mean control values were 293 276 pg IL-8 mg^{-1} and those for 0·1, 1·0 and 5·0 μM meloxicam were 387 891, 222 181 and 224 204 pg IL-8 mg^{-1}, respectively.

Discussion

The principal conclusions from this study are that meloxicam has no significant inhibitory effects on the synthesis or degradation of radiosulphate-labelled proteoglycans in cartilage from man or pig, whether incubated in the presence or absence of IL-1α, whereas indomethacin reduced proteoglycan synthesis (Figs 1 and 2); that meloxicam seemed to increase qualitatively the proportion of the high-molecular weight fraction of ${}^{35}SO_4^{2-}$ proteoglycans synthesized by human cartilage in contrast with indomethacin which reduced the proportion of high-molecular weight proteoglycans (Fig. 3); that whereas PGE_2 production was reduced by low concentrations of meloxicam (Table 1), the drug did not affect the

Table 1. Effects of meloxicam on production of IL-1 and IL-6 compared with PGE_2 production by human synovium.

Treatment		Interleukin-1 bioactivity* (counts min^{-1} (mg protein)$^{-1}$)	Interleukin-1α ELISA† (pg (mg protein)$^{-1}$)	Interleukin-6 ELISA* (ng (mg protein)$^{-1}$)	Prostglandin E_2* (pg (mg protein)$^{-1}$)
Group A					
Control		25120 ± 1206 (3)	539	8712 ± 2143 (5)	
Meloxicam	0·1 μM	36394 ± 8676 (3)	378	14979 ± 1949 (5)‡	
	4·0 μM	28701 ± 5104 (4)	806	13690 ± 1428 (5)‡	
	10·0 μM	21841 ± 3768 (3)	678		
Hydrocortisone	10 μM	10411 ± 3124 (4)§		4043 ± 712 (5)§	
Group B					
Control		4549 ± 1906 (5)	204		219 ± 52 (5)
Meloxicam	0·1 μM			14 ± 2·3 (5)§	
	4 μM	5963 ± 2576 (5)	281		23·7 ± 7·1 (5)§
	10 μM	6661 ± 1437 (5)	244		19·0 ± 5·2 (5)§
Control		20248 ± 6820 (5)			
Indomethacin	10 μM	19430 ± 6261 (5)			
	100 μM	68852 ± 5762 (5)‡			
Group C	In PEG-8000 (same tissue samples as in Group A) treated culture media (removes IL-1 inhibitors)				
Control		33555 ± 3052 (3)			
Meloxicam	0·1 μM	46130 ± 12175 (3)			
	4·0 μM	34891 ± 5627 (4)			
	10·0 μM	25306 ± 4499 (3)			
Control		1157 ± 146 (3)			
Meloxicam	10 μM	1846 ± 1437 (3)			
Control		464 ± 264 (5)		1887 ± 387 (5)	
Indomethacin	10 μM	436 ± 61 (5)		3503 ± 610 (5)‡	
	100 μM	684 ± 112 (5)‡		5302 ± 692 (5)‡	

*Values are means ± s.d.; the numbers in parentheses indicate the number of samples. †Assayed in duplicate. ‡Significant increase (Student's *t*-test; $P < 0.05$ compared with control). §Significant reduction (Student's *t*-test; $P < 0.05$ compared with control). The same lack of effect was evident in data from on other samples.

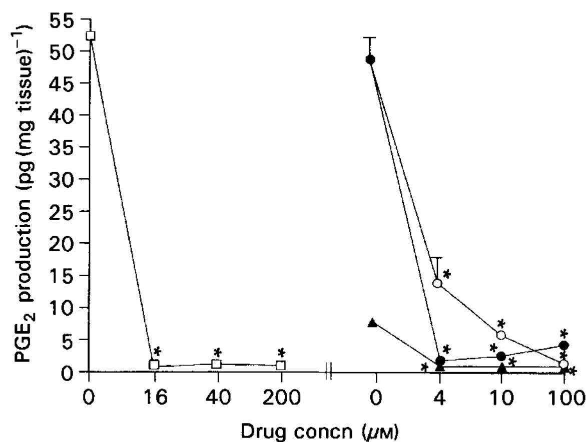

FIG. 4. Effects of meloxicam, compared with other NSAIDs, on PGE_2 production by human synovial tissues. (a) $*P < 0.001$ compared with control (n = 5 group^{-1}). (b) $*P < 0.001$ compared with control, $†P < 0.05$ compared with control (n = 5 group^{-1}).

production of the pro-inflammatory cytokines, interleukins 1 and 8 at drug concentrations (≤ 4 μM) considered to be within the range expected in synovial fluids or synovia during therapy (Degner et al 1994); and that meloxicam, like indomethacin, stimulated synovial production of IL-6. This cytokine is considered to have part anti-inflammatory activity because of induction of acute-phase proteins (Whicher & Westacott 1992; Graeve et al 1993) even though the production of some of these proteins is also a reflection or manifestation of the synthesis of the pro-inflammatory cytokines, IL-1 and tumour necrosis factor-α (Whicher & Westacott 1992). IL-6 stimulates production of tissue inhibitor of metalloproteinases-1, but not the metalloproteinases (Sato et al 1990) implying that this cytokine also has inhibitory effects on degradative reactions in joints.

The stimulation by indomethacin of IL-1 production by human synovia (Table 1) has also been observed in human monocytes (Bonta & Elliott 1992). This has been ascribed to the effects of this drug on stimulation of leukotriene B_4 production caused by diversion of arachidonate metabolism after cyclooxygenase inhibition (Bonta & Elliott 1992). Inhibitors of leukotriene production are known to block production of IL-1 (Rainsford et al 1996) thus supporting the concept. Why meloxicam, also a potent inhibitor of cyclooxygenase activity, does not cause stimulation of IL-1 is not clear. It might be that stimulation of IL-1 production is related to inhibition of cyclooxygenase-1, because indomethacin is known to be a more potent inhibitor of this isoform than of the inducible cyclooxygenase-2 (Vane & Botting 1995). As meloxicam is a selective inhibitor of cyclooxygenase-2 relative to cyclooxygenase-1 (Vane & Botting 1995) it might be argued that the stimulation of IL-1 production is possibly a phenomenon related to inhibition of cyclooxygenase-1 but not cyclooxygenase-2. Whatever the mechanism, it is likely that stimulation of IL-1 production by indomethacin could potentiate the cartilage destruction observed in osteoarthritis (Bonta & Elliott 1992; Rainsford et al 1992).

Inhibition of cartilage proteoglycan metabolism by indomethacin and some other NSAIDs has been proposed as a

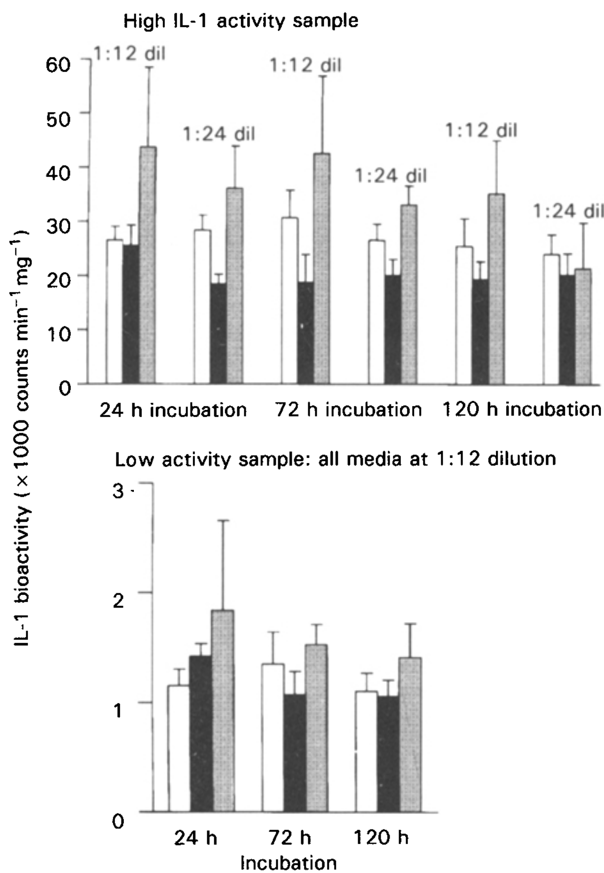

FIG. 5. The effect of time of incubation with meloxicam, intrinsic bioactivity and media dilution on production of IL-1-like activity by human synovial tissue in organ culture. No significant differences were observed among the various treatments (Student's t-test, $P > 0.05$).

reason for the apparent acceleration of cartilage destruction and joint-space narrowing observed with these drugs (Dingle 1992; Jones & Doherty 1992; Rainsford et al 1992). The lack of qualitative effect of meloxicam on proteoglycan synthesis and synthesis of high- compared with low-molecular weight proteoglycans (Figs 1–3), combined with the above mentioned actions on interleukin production and the potent inhibitory actions on polymorphonuclear leucocyte oxyradical production (Rainsford et al 1997), might all contribute to the potential to limit cartilage destruction especially in comparison with drugs such as indomethacin.

Claims have been made, on the basis of studies of proteoglycan synthesis in-vitro, that some NSAIDs (e.g. tiaprofenic acid) might induce cartilage protection in patients with osteoarthritis receiving these drugs (Jones & Doherty 1992; Rainsford 1996). Such claims have proven controversial, especially when there is little or no evidence from long-term studies in patients with osteoarthritis (Jones & Doherty 1992; Rainsford 1996). Therefore, caution must be expressed in extrapolating observations from in-vitro systems until they are confirmed from long-term in-vivo studies in patients (Rainsford 1996). This is also true for the results of the current study but these give impetus for further studies to be performed on patients with osteoarthritis.

From a technical viewpoint, particular care has been given in these studies to take into account the intrinsic variability that exists in human tissues derived from subjects with osteoarthritis who have received various drug treatments and have varying pathology. Thus, the discrimination of drug effects on cartilage proteoglycan and interleukin metabolism in high- compared with low-activity samples (Table 1; Figs 1a–1e, 5a and 5b) shows that there is considerable consistency of drug effects irrespective of differences in the rates of metabolism of proteoglycans or the interleukins in these samples.

In conclusion, these studies show that meloxicam, like some but not all other NSAIDs, has a profile with no evident untoward biochemical action on cartilage function, indicating that it might be worth examining the cartilage of osteoarthritis patients receiving this drug for long-term pain relief.

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