J. Pharm. Pharmacol. 1989, 41: 112–117
Received August 2, 1988

© 1989 J. Pharm. Pharmacol

Effects of Anti-inflammatory Drugs on Interleukin 1-induced Cartilage Proteoglycan Resorption In-vitro: Inhibition by Aurothiophosphines but no Influence from Perturbed Eicosanoid Metabolism

K. D. RAINSFORD

Anti-inflammatory Research Unit, Strangeways Research Laboratory, Cambridge, CB1 4RN, UK

Abstract—A range of anti-inflammatory drugs having varying effects on eicosanoid metabolism and other actions was studied for their potential to inhibit α-interleukin 1 (IL-1)-induced cartilage proteoglycan resorption in-vitro. No significant effects on resorption were observed with inhibitors of cyclo-oxygenase, lipoxygenase or mixed inhibitors of both these enzymes, and no influence on IL-1 effects was observed with added eicosanoids. Among the clinically used disease modifying anti-arthritic agents, only auranofin and the immunoregulatory agent, tilomisole, were found effective in inhibiting resorption. Some auranofin analogues having chloride or nitrate leaving groups that inhibit DNA polymerase-α were found to be potent inhibitors of IL-1 induced resorption.

Interleukin 1 (IL-1) is present in inflamed synovial fluids (Bendtzen et al 1985) and in various forms activates chondrocytes and induces the resorption of cartilage proteoglycans in-vitro (Saklatvala et al 1984; Krakauer et al 1985) and directly or indirectly by inducing leucocyte emigration in-vivo (Pettipher et al 1986; Dingle et al 1987). During exposure to IL-1 chondrocytes produce appreciable quantities of prostaglandin (PG) E_2 (McGuire-Goldring et al 1984; Carroll 1986; Chang et al 1986), from direct activation of phospholipase A_2 (Chang et al 1986). This activation of the pathways of eicosanoid metabolism suggests that there may be potential for pharmacological control of IL-1 induced cartilage resorption by those anti-inflammatory drugs which affect arachidonic acid release and metabolism.

Previously, chloroquine and related antimalarial drugs were found to inhibit IL-1 induced cartilage resorption in-vitro (Rainsford 1986). Since these drugs inhibit phospholipase A_2 it seemed possible that these drugs might exert their inhibitory actions on cartilage resorption, in part, by blocking release of arachidonate. However, conventional cyclo-oxygenase (CO) inhibitors (e.g. aspirin, diclofenac, indomethacin, piroxicam) have been found ineffective in preventing cartilage resorption by IL-1 (Rainsford 1985). Alternatively, IL-1-induced cartilage resorption might be controlled by inhibiting the alternate lipoxygenase(s) (LO) pathway(s) of arachidonic acid metabolism or by a combination of inhibition of CO and LO activities.

Thus in the present studies, the effects were studied of (a) a range of experimental LO, mixed CO/LO inhibitors and phospholipase A_2 inhibitors, and (b) the influence of addition of CO and LO products of eicosanoid metabolism on IL-1 induced cartilage resorption in-vitro. Furthermore, since many newer non-steroidal anti-inflammatory drugs (NSAIDs) and disease modifying anti-arthritic drugs (DMARDs) have little or no influence on arachidonic acid metabolism but, as in the case of D-penicillamine, can inhibit cartilage resorption in-vivo (Sedgwick et al 1984), these agents were examined for potential to directly inhibit IL-1 induced cartilage proteoglycan resorption in-vitro.

Correspondence to: K. D. Rainsford, Arthritis Center and Dept. of Biomed. Sci. (HSC-2F9), McMaster University, Faculty of Health Sciences, 1200 Main Street W., Hamilton, Ontario, Canada.

Materials and Methods

The methods employed are essentially those as described in the previous studies (Rainsford 1986). Thus, bovine nasal septum cartilage from freshly (< 1h) slaughtered adult cattle was dissected under sterile conditions and transverse slices (1·5 mm thick) cut from which discs (2 mm in diameter) were obtained with the aid of a sterile leather punch. The cartilage discs were pre-incubated for 2 days in Microtiter 92 well dishes in the presence of 200 μL Dulbecco's modified Eagle's medium (DMEM) containing penicillin (200 u mL^{-1}), streptomycin (200 μ mL^{-1}), and 5% foetal calf serum (FCS) in an atmosphere of 5% CO_2 in air (Saklatvala et al 1984). The medium was then changed and the drugs or solvent mixtures (see Table 1), together with chromatofocussed pI 4·8 porcine leucocyte α-IL-1 (gift of Dr J. Saklatvala, Strangeways Research Laboratory, prepared as described by Saklatvala et al 1984), or human recombinant r-α-IL-1 (gift of National Institute for Biological Standards and Control, Potters Bar, Herts., UK Code No. 86/632, or purchased from Genzyme Corp., Boston, MA, USA) were added. Sufficient IL-1 was added to give at least a 80–90% degradation of the cartilage compared with that in control cultures without IL-1. Incubations with drug or solvent mixtures were performed in octuplicate for 4 days. Control cultures (without IL-1) had drugs alone or solvent mixtures added. Drug or solvent cytotoxicity was determined by comparing the release of glycosaminoglycans (GAGs) (see below for methods) from those cultures to which the drugs or solvent mixtures had been added, with GAG release in controls (in DMEM ± 5% FCS).

Cartilage proteoglycan degradation was determined by measurement of the content of GAGs in the media compared with that in papain-digested cartilage (Saklatvala et al 1984). The assay of GAGs was performed using dimethyl-methylene blue (Serva) technique. None of the drugs examined affected this assay.

Drugs

The following drugs were generously donated as shown in brackets:

Allopurinol and BW-755c (3-amino-1-(3-trifluoromethylphenyl)-2-pyrazoline HCl (Dr P. J. McHale, Wellcome Research Laboratories, Beckenham, Kent); amfenac sodium (A. H. Robins Co. Ltd., Richmond VA, USA); auranofin (SKF-39162) (Mr M. Massey-Stewart, Smith, Kline & French Labs., Welwyn Garden City); clobuzarit (Clozic, Dr M. E. Billingham, ICI Pharmaceutics Div., Alderley Park); cyclosporine A (Sandoz AG, Basel, Switzerland); ebselen (Dr M. J. Parnham, A. Natterman GmbH, Köln, FRG); etofenamate (Troponwerke, GmbH & Co KG, Köln, FRG); OKY-046 (lot 57-10-1; (E)-3-(4-(1-imidazolylmethyl)phenyl)-2-propionoate sodium salt), ONO-3144 (lot J3001; 2-aminomethyl-4-t-butyl-6-propionyl phenol HCl), prostaglandins E_2, $F_{2\alpha}$ and B_2, thromboxane B_2 (lot nos. 93, 157, 13 and 5, respectively (Drs Keith Crowshaw and M. Tsuboshina, ONO Pharmaceutical Co., Osaka, Japan); flufenisal and L-651,896 (2,3-dihydro-6-(3-(2-hydroxymethyl) phenyl-2-propenyl)-5-benzofuranol: Merck, Sharp & Dohme, Rahway, NJ, USA); ketoprofen (May & Baker Ltd., Dagenham); leukotrienes B_4, C_4 and D_4, 5-hydroxeicosatetraenoic acid (5-HETE), L-640,035 (3-hydroxymethyldibenzo [b,f]-thiepin-5,5-dioxide), L-636,499 (dibenzo [b,f]-thiepin-3-carboxylic acid, 5,5-dioxide), (Drs J. Rokach and A. W. Ford-Hutchinson, Merck-Frosst Canada Inc., Pointe-Claire Dorval, Canada); leukotrifina (Ellem Indenstria Farmaceutica SpA, Milan; levamisole, R-9280 (OMPI, Dr. M. B. Emanuel, Janssen Pharmaceutical Ltd., Wantage); methotrexate (Lederle Laboratories, Gosport, Hampshire); nabumetone (Beecham Research Laboratories, Brentford, Middlesex); oxaprozin, tilomisole (=Wy 18,251), Wy-45,637, (α-hydroxy-5-oxo-2-phenyl-1-pyrazolidineacetic acid) Wy-46,679, (*N*-[3-(2-benzothiazolylmethoxy)phenyl])-ethanesulfonamide), Wy-46,904, (3-(2-benzothiazolylmethoxy)-*N*-hexylbenzenamine, dihydrobromide), Wy-46,905, (2-[(3-bromophenoxy)methyl]quinoline) (Drs J. Chang and A. J. Lewis, Wyeth Laboratories Inc., Radnor, PA, USA); D-penicillamine (Carter-Wallace Laboratories, Cranbury, NJ., USA); Penicillamine cysteine (Lilly Research Centre Ltd., Windlesham, Surrey); REV-5901 (2-3′-(1′-hydroxyhexyl)-phenoxy)-methyl-quinoline, Revlon Health Care Group, Tuckahoe, NY, USA; SKF-36,914 (chloro(triethyl phosphine)gold), SKF-40,117 (tris(dimethylaminophosphine)gold chloride) SKF-85,626 (2,3,4,6-tetra-*O*-acetyl-1-B-D-glucopyranosyl-bis-[(triethylphosphine)-aurio] sulphonium chloride), SKF-100,336 (2,3,4,6-tetra-*O*-acetyl-2-B-D-glucopyranosyl-bis-[(triethylphosphine)-aurio] sulphonium nitrate) (Dr H. S. Allaudeen, Smith Kline & French Labs., Swedeland, PA, USA), sulphasalazine, sulphapyridine and 4-aminosalicylic acid (Mr A. D. Turner, Pharmacia Ltd., Milton Keynes); tenoxicam (Dr N. Rogers, Roche Products Ltd., Welwyn Garden City).

Azathioprine, benzimidazole, cyclophosphamide, and penicillamine disulphide were purchased from Sigma, Poole, Dorset, while sodium aurothiomalate and thiomalic acid (mercaptosuccinic acid) were purchased from Aldrich, Gillingham, Dorset.

All of the drugs except ONO-3144, MK-447 and the auranofin analogues were first dissolved in ethanol then DMEM/FCS added to give a final concentration of 0·1% ethanolic solutions. This concentration of ethanol does not affect the responses of the cartilage to IL-1. The other compounds were dissolved direct in the culture medium.

Results and Discussion

The results in Table 1 show that none of the drugs which are CO inhibitors (group I), LO inhibitors (group III) thromboxane synthesis inhibitors/antagonists or prostaglandin antagonists (group IV) exhibited significant inhibitory effects on α-IL-1 induced cartilage proteoglycan resorption in-vitro. The lack of effects of the range of CO inhibitors of which ONO-3144 is also an antoxidant, is in agreement with the inactivity observed previously of other more commonly used NSAIDs (Rainsford 1985). Two of the mixed CO/LO inhibitors, etofenamate and L-651,896 (group II) exhibited some inhibitory effects at the highest concentration (100 μg mL^{-1}) studied. The inhibitory effects of these agents can hardly be considered potent especially in relation to their range of plasma concentrations in therapy nor that in comparison with anti-malarials as previously reported (Rainsford 1986).

No influence on α-IL-1 induced resorption was observed with prostaglandin (PG) E_2, $F_{2\alpha}$, D_2, B_2 or 6-keto $PGF_{1\alpha}$ (at concentrations of 500, 50, 5 or 0·5 mg mL^{-1}) leukotrienes (LTs) B_4, C_4, D_4 or 5-hydroxyeicosatetraenoic acid (5-HETE) at concentrations of 50, 5, 0·5 or 0·05 mg mL^{-1}), nor that of the mixture of PGE_2 (500 mg mL^{-1}) with LTB_4 (50 mg mL^{-1}) (data not shown). None of the PGs, LTs or 5-HETE affected the concentration of proteoglycans in cartilage discs nor the basal release of GAGs in the absence of α-IL-1 (data not shown) showing that these eicosanoids do not influence resorption.

These results show that alteration in the production or actions of the eicosanoids clearly does not appreciably influence the resorptive effects of α-IL-1 on cartilage chondrocytes. It could be argued that the effects of etofenamate and of L-651,896 represent notable departures from this conclusion. However, the high concentrations at which inhibitory effects were observed with these two agents is suggestive of lack of specific actions.

Of the DMARDs only auranofin and tilomisole (Wy 18,251), a weak inhibitor of CO with immunopharmacological activity (Colot & Miksche 1982–83; Lewis et al 1982–83) were inhibitory. The effects of tilomisole were however, only evident at the highest concentration and the relevance of this in therapy is difficult to discern.

The lack of effects of the gold thiomalate compounds on α-IL-1 induced cartilage resorption (Group VIA) contrasts with the reported inhibitory effects of these compounds on IL-1 induced thymocyte mitogenesis (Drakes et al 1987).

In view of the inhibition of IL-1 induced resorption by

Table 1. Effects of anti-inflammatory drugs on α-Interleukin 1 induced cartilage proteoglycan resorption in-vitro

Group	Drug	Concn	Proteoglycan resorption % GAGs released (mean ± s.d.) With IL-1	No IL-1
I.	Potent cyclo-oxygenase inhibitors (not previously studied)			
	Amfenac sodium	100 μM	92·0 ± 2·3	15·2 ± 4·4
		10 μM	92·3 ± 3·9	19·8 ± 7·1
	Flufenisal	100 μM	93·3 ± 5·2	18·9 ± 7·8
		20 μM	96·3 ± 4·9	19·5 ± 3·1
	Ketoprofen	500 μM	89·9 ± 19·4	12·7 ± 3·7
		100 μM	40·2 ± 41·5	10·0 ± 2·8
	ONO-3144	100 μM	85·6 ± 27·7	18·6 ± 5·5
	Tenoxicam	100 μM	92·2 ± 0·8	19·9 ± 6·6
		20 μM	93·8 ± 0	14·3 ± 12·1
	Control		96·2 ± 2·3	20·9 ± 6·5
II.	Mixed cyclo-oxygenase/lipoxygenase inhibitors			
	BW-755c	100 μM	73·4 ± 24·9	17·1 ± 5·3
		10 μM	94·8 ± 0·9	13·2 ± 2·8
		5 μM	82·3 ± 8·9	13·5 ± 6·7
	Etofenamate	100 μg mL⁻¹	21·7 ± 18·0*	12·8 ± 6·3
		20 μg mL⁻¹	95·8 ± 6·4	19·2 ± 1·8
	L-651,896	100 μg mL⁻¹	14·0 ± 16·3*	10·6 ± 2·2
		20 μg mL⁻¹	70·9 ± 35·4	15·1 ± 3·2
	MK-447	500 μM	84·9 ± 22·5	10·9 ± 6·3
	Sulphasalazine (SASP)	100 μM	92·4 ± 4·3	19·3 ± 7·7
		20 μM	99·2 ± 2·1	30·4 ± 6·2
	5-Aminosalicylic acid (SASP metabolite)	100 μM	71·6 ± 33·8	33·2 ± 1·7
	Sulphapyridine	100 μM	96·9 ± 3·8	23·4 ± 1·9
	(SASP metabolite)	20 μM	94·2 ± 3·8	19·3 ± 7·7
	Control		94·9 ± 1·0	11·9 ± 4·6
III.	5-Lipoxygenase inhibitors/LT antagonists			
	REV-5901	100 μM	88·7 ± 5·4	23·0 ± 3·7
		10 μM	86·7 ± 9·1	15·3 ± 4·5
		5 μM	86·3 ± 13·8	14·9 ± 8·1
	Ebselen	100 μM	92·5 ± 1·9	16·4 ± 2·2
		10 μM	95·0 ± 0·5	14·8 ± 5·0
	Isomoxole	100 μM	89·0 ± 24·6	17·0 ± 4·7
		10 μM	90·9 ± 17·8	17·1 ± 7·1
	Wy-45,637	100 μM	100·0 ± 13·2	23·4 ± 2·1
		20 μM	100·8 ± 25·0	
	Wy-46,679	100 μM	111·3 ± 14·9	36·2 ± 19·4
		20 μM	98·0 ± 23·9	
	Wy-46,904	100 μM	69·4 ± 14·0	18·3 ± 6·0
		20 μM	101·1 ± 14·5	
	Wy-46,905	100 μM	88·8 ± 11·1	19·7 ± 8·5
		20 μM	101·8 ± 37·4	
IV.	Thromboxane synthesis inhibitors/antagonists and prostaglandin antagonists			
	Benzamidazole	100 μM	91·7 ± 1·9	16·0 ± 3·0
		10 μM	95·5 ± 0·8	16·0 ± 2·9
	L-640,035	100 μg mL⁻¹	74·9 ± 36·5	25·3 ± 6·2
		20 μg mL⁻¹	103 ± 42·7	28·6 ± 11·4
	L-636,499	100 μg mL⁻¹	79·8 ± 29·8	16·1 ± 6·9
	(acetic acid metabolite of L-640,035)	20 μg mL⁻¹	62·3 ± 7·5	13·8 ± 4·2
	OKY-046	30 μg mL⁻¹	81·5 ± 15·7	17·4 ± 6·5
	Control		89·8 ± 4·2	27·7 ± 8·2
V.	NSAIDs with weak or no effects on eicosanoid metabolism			
	Allopurinol	1000 μM	84·6 ± 18·4	29·2 ± 11·4
		10 μM	109·3 ± 36·4	26·4 ± 4·6
	Clobuzarit	130 μM	79·9 ± 26·7	16·2 ± 6·4
	(Clozic)	13 μM	79·5 ± 13·5	15·5 ± 1·1
		6·5 μM	94·9 ± 1·6	19·1 ± 5·5
	Nabumetone	500 μM	68·5 ± 35·6	18·8 ± 8·8
		100 μM	64·1 ± 26·1	14·5 ± 5·5
	Control		94·1 ± 1·0	15·3 ± 4·5
	Oxaprozin	100 μM	100·5 ± 29·2	30·7 ± 12·4
		100 μM	115 ± 27·8	27·1 ± 14·9
		100 μM	54·6 ± 19·7	50·5 ± 26·9

Table 1 (*cont.*)

Group	Drug	Concn	Proteoglycan resorption % GAGs released (mean ± s.d.) With IL-1	No IL-1
	Tilomisole Expt 1	100 μM	49·0 ± 20·8*	18·5 ± 5·3
	(Wy-18,251)	10 μM	70·7 ± 16·9	15·3 ± 5·4
	Expt 2	100 μM	53·8 ± 28·2*	23·4 ± 6·7
		10 μM	89·3 ± 21·3	19·3 ± 6·8
	Wy-41,770	100 μM	59·5 ± 26·1	15·6 ± 7·9
		10 μM	60·0 ± 21·7	21·2 ± 9·0
	Control		90·6 ± 6·3	20·4 ± 7·0
VI.	Disease-modifying anti-rheumatic drugs			
	A. Thiolates			
	Auranofin	100 μM	12·4 ± 5·7*	16·7 ± 7·3
		20 μM	16·0 ± 8·6*	18·8 ± 7·2
		10 μM	38·6 ± 22·0*	20·4 ± 5·7
		5 μM	63·0 ± 28·3*	26·9 ± 21·1
		1 μM	88·1 ± 2·6	13·8 ± 2·1
		0·1 μM	87·1 ± 3·9	11·0 ± 2·9
		10 nM	92·2 ± 1·6	10·4 ± 4·9
		1·0 nM	92·0 ± 2·6	22·0 ± 8·8
	Levamisole	100 μM	94·4 ± 1·5	23·3 ± 4·7
		10 μM	95·3 ± 0·6	23·1 ± 5·4
	OMPI (metabolite of	100 μM	94·6 ± 1·2	20·9 ± 8·9
	levamisole)	10 μM	93·5 ± 2·1	21·6 ± 2·3
	D-Penicillamine	100 μM	91·6 ± 8·0	14·8 ± 5·1
		20 μM	89·3 ± 8·5	20·9 ± 30·0
	L-Penicillamine	100 μM	95·6 ± 8·0	9·9 ± 6·7
		20 μM	90·4 ± 10·0	24·8 ± 25·0
	Penicillamine	100 μM	91·4 ± 1·5	13·6 ± 7·2
	cysteine	20 μM	82·3 ± 8·6	17·1 ± 5·1
	Penicillamine	100 μM	87·9 ± 5·6	13·2 ± 3·6
	disulphide	20 μM	80·4 ± 20·5	16·1 ± 4·8
	Sodium auro-	100 μM	93·7 ± 1·1	28·3 ± 6·4
	-thiomalate	10 μM	94·9 ± 1·2	31·4 ± 12·4
	Control		94·9 ± 0·6	23·6 ± 11·4
	Thiomalate	100 μM	94·7 ± 1·4	29·4 ± 9·6
		10 μM	85·2 ± 12·7	28·8 ± 3·4
	B. Cytotoxic or immunoregulant agents			
	Azathioprine	50 μM	63·6 ± 27·6	18·5 ± 2·4
		10 μM	96·9 ± 23·4	14·3 ± 1·0
		1 μM	78·8 ± 28·5	13·8 ± 2·2
	Cyclophosphamide	50 μM	66·5 ± 32·6	11·3 ± 6·8
		10 μM	94·8 ± 26·3	23·9 ± 7·8
		5 μM	100 ± 28·1	22·0 ± 2·3
		1 μM	78·8 ± 25·0	26·1 ± 4·0
	Cyclosporin A	200 μg mL^{-1}	30·4 ± 15·5*	13·6 ± 3·2
		20 μg mL^{-1}	55·8 ± 27·8	24·0 ± 8·9
	Leukotrifina	150 μg mL^{-1}	80·9 ± 17·4	36·2 ± 11·6
		50 μg mL^{-1}	87·0 ± 7·2	25·0 ± 10·7
	6-Mercaptopurine	100 μM	94·8 ± 1·5	41·9 ± 27·0**
		20 μM	91·3 ± 2·7	46·0 ± 24·0**
		10 μM	94·2 ± 1·8	52·6 ± 26·4**
	Methotrexate	100 μM	81·7 ± 11·8	12·7 ± 4·2
		10 μM	84·7 ± 11·9	11·5 ± 2·5
		5 μM	81·3 ± 16·9	12·8 ± 5·3
	Control		90·6 ± 6·3	20·4 ± 7·0

Cartilage proteoglycan resorption was determined by assaying the glycosaminoglycan (GAG) content in the media and the cartilage disc (after papain digestion);

$$\text{the percent GAGs} = \frac{\text{GAGs in Medium}}{\text{GAGs in Medium} + \text{disc}} \times \frac{100}{1}$$

*Denotes significant reduction (Student's *t*-test for unpaired data, $P < 0{\cdot}05$, N = 8/group) c.f. controls. The control values in groups III are shown in group VI. Some drugs e.g. SASP and ebselen, may have more complex actions on arachidonic acid and oxyradical metabolism than indicated by the categories above. The high basal release of GAGs with 6-mercaptopurine** is due to a cytotoxic action of this drug on chondrocytes.

certain anti-tumour antibiotics (e.g. echinomycin) with inhibitory actions on DNA polymerases (Rainsford 1985), it was decided to explore the effects on IL-1 resorption by some auranofin analogues which have been reported to inhibit DNA polymerase (Allaudeen et al 1985). The results in Table 2 show that of these gold compounds studied, each inhibited α-IL-1 induced cartilage proteoglycan resorption in a concentration-dependent fashion. With concern that there may be unspecific, possibly toxic effects on overall protein synthesis by these gold compounds, their effects on the

Table 2. Effects of aurothiophosphine compounds on Interleukin-1-induced cartilage proteoglycan resorption and [^{3}H] leucine incorporation in-vitro.

Drug	Conc(μg mL^{-1})	Proteoglycan resorption % GAGs released (mean ± s.d.)		IC50 (μg mL^{-1})	L-[4,5]-^{3}H-Leucine Incorporation d min^{-1} mg^{-1} d.wt. (mean ± s.d.)	
		With IL-1	No IL-1		With IL-1	No IL-1
Gold compounds						
SKF-36,914	20	29·2 ± 4·4*	17·6 ± 3·0			
	4·0	75·7 ± 19·9	14·4 ± 3·1	5·2	50 591 ± 6154	48 488 ± 8148
	2·0	82·2 ± 13·0	29·4 ± 13·5			
	0·8	88·2 ± 8·0	12·4 ± 6·2			
	0·4	90·0 ± 4·7	14·8 ± 1·9			
SKF-40,117	20	22·8 ± 8·1*	27·4 ± 16·0			
	4·0	42·5 ± 21·7*	18·1 ± 3·1		40 349 ± 3909	44 954 ± 9320
	2·0	80·7 ± 4·3	17·5 ± 1·6	3·5		
	0·8	84·1 ± 8·7	18·0 ± 1·2			
	0·4	87·7 ± 6·5	19·6 ± 4·4			
SKF-85,626	20	27·5 ± 9·5*	20·7 ± 3·8			
	4·0	12·3 ± 1·4*	14·6 ± 2·5	1·5	30 301 ± 2853	36 281 ± 18 515
	2·0	28·6 ± 11·2	23·1 ± 6·2			
	0·8	80·9 ± 23·2	12·8 ± 16·0			
SKF-199,336	0·4	78·5 ± 18·6	12·1 ± 2·1			
Control		94·9 ± 0·6	23·6 ± 11·4		40 622 ± 11 970	37 465 ± 11 366
SKF-100,336	20	22·4 ± 9·1*	24·9 ± 9·6			
	4·0	10·5 ± 1·4	14·4 ± 2·5	1·4	41 323 ± 6936	43 936 ± 269
	2·0	28·9 ± 19·5*	24·4 ± 16·0			
	0·8	83·3 ± 6·6	19·2 ± 1·3			
	0·4	90·8 ± 1·8	17·2 ± 5·6			

*Statistically significant reduction (student's *t*-test) $P < 0·05$, N = 8/group for proteoglycan resorption studies; N = 4/group for [^{3}H]leucine incorporation.

incorporation of radio-actively labelled leucine were determined. The results (Table 2) show that at concentrations at which inhibitory effects were shown with the gold compounds no inhibition of overall protein synthesis was observed suggesting that non-specific effects were not involved in the apparent inhibitory actions of these agents on IL-1 induced resorption. Moreover when the cartilage explants were cultured for 24 h with auranofin, and then washed free of drug (3 × with sterile phosphate buffered saline) before adding α-IL-1, it was found that the release of GAGs was not significantly depressed in those cultures exposed to < 20 μM of the drug. This suggests that the effects of auranofin up to this concentration are reversible and may therefore be independent of any cytotoxic actions.

It appears that the inhibition of IL-1 induced resorption by the aurothiophosphine compounds may relate to their effects as inhibitors of DNA polymerase-α rather than to their more generalized capacity as inhibitors of DNA replication. Thus comparison of the IL-1 resorption data with data of Allaudeen et al (1985) of the two DNA enzymic activities shows that auranofin which had an IC50 of 7 μM in the resorption assay (Table 1) has inhibitory effects on DNA polymerase-α which are appreciably less than those of the aurothiophosphines listed in Table 2. These latter compounds have inhibitory effects on DNA polymerase-α in the range of 2·5–5·5 μM) and, coincidently, are all inhibitors of the IL-1-induced resorption in cartilage in the same order of magnitude. Comparison with these drug effects on DNA replication (Allaudeen et al 1985) shows that auranofin is approximately equipotent with all the other drugs. The reason for this differing activity may be related to the presence of the chloride or nitrate groups on the four inhibitors of IL-1 induced resorption shown in Table 2, these being less so with auranofin. These four compounds have been proposed to be better inhibitors of the DNA polymerase activity because of the ability of the chloride and nitrate moieties to act as better leaving groups (Allaudeen et al 1985).

The lack of effects of the thiolate compounds compared with that of the aurothiophosphines may be due to their rates of uptake into cells or in the case of free thiol compounds their ability to bind to natural thiols before reaching the site of action on DNA replicating systems; thiol groups on DNA polymerase having been suggested as sites of action for inhibitory effects of the autothiophosphines (Allaudeen et al 1985). Aurothiophosphines may represent leads for the development of specific inhibitors of the synthesis of those intracellular enzymes or components responsible for cartilage resorptive processes.

Acknowledgements

My thanks to Miss Alison Davies for expert technical assistance. I also thank those companies who most generously donated compounds for this study. These studies were supported by a grant from the Wolfson Trust (UK).

References

Allaudeen, H. S., Snyder, R. M., Whitman, M. H., Crooke, S. T., (1985) Effects of co-ordinated gold compounds on *in vitro* and *in situ* DNA Replication. Biochem. Pharmacol. 34: 3243–3250

Bendtzen, K., Petersen, J., Halkjaer-Kristensen, J., Ingelmann-Hansen, T. (1985). Interleukin-1-like Activities in Synovial Fluids of Patients with Rheumatoid arthritis. Rheumatol. Int. 5: 79–82

Carroll, G. J. (1986) A Study of the Effects of Catabolin on Cyclic Adenosine Monophosphate Biosynthesis and Prostaglandin E_2 Secretion in Pig Articular Chondrocytes. Br. J. Rheumatol. 25: 359–365

Chang, J., Gilman, S. C., Lewis, A. J. (1986) Interleukin 1 activates phospholipase A_2 in rabbit chondrocytes: a possible signal for IL 1 action. J. Immunol. 136: 1283–1287

Colot, M., Misksche, M. (1982–83) Influence of thia-benzimidazole Wy 18251 of cancer patients' lymphocyte reactivity. J. Immunopharmacol. 4: 367–376

Dingle, J. T., Page-Thomas, D. P., King, B., Bard, D. R. (1987) *In vivo* studies on articular tissue damage mediated by catabolin/interleukin 1. Ann. Rheum. Dis. 46: 527–533

Drakes, M. L., Harth, M., Galsworthy, S. B., McCain, G. A. (1987) Effects of gold on the production of and response to human interleukin-1. J. Rheumatol. 14: 1123–1127

Krakauer, T., Oppenheim, J. J., Jasin, H. E. (1985) Human interleukin 1 mediates cartilage degradation. Cellular Immunol. 91: 92–95

Lewis, A. J., Parker, J., Di Luigi, J., Datko, L. J., Carlson, R. P. (1982–83) Immunomodulation of delayed hypersensitivity to methylated bovine serum albumin (MBSA) in mice using subliminal and normal sensitization procedures. J. Immunopharmacol. 3: 289–307

McGuire-Goldring, M. B., Meats, J. E., Wood, D. D., Ihrie, E. J., Ebsworth, N. M., Russell, R. G. G. (1984) *In vitro* activation of human chondrocytes and synoviocytes by a human interleukin-1-like factor. Arth. Rheum. 27: 654–662

Pettipher, E. R., Higgs, G. A., Henderson, B. (1986) Interleukin 1 induces leukocyte infiltration and cartilage proteoglycan degradation in the synovial joint. Proc. Natl. Acad. Sci. 83: 8749–8753

Rainsford, K. D. (1985) Preliminary investigations on the pharmacological control of catabolin-induced cartilage destruction *in vitro*. Agents Actions 16: 55–57

Rainsford, K. D. (1986) Effects of antimalarial drugs on interleukin 1-induced cartilage degradation in-vitro. J. Pharm. Pharmacol. 38: 829–833

Saklatvala, J., Pilsworth, L. M. C., Sarsfield, S. J., Gavrilovic, J., Heath, J. K. (1984) Pig catabolin is a form of interleukin 1. Cartilage and bone resorb, fibroblasts make prostaglandin and collagenase, and thymocyte proliferation is augmented in response to one protein. Biochem. J. 224: 461–466

Sedgwick, A. D., Moore, A. R., Sim, Y. M., Al-Duaij, A. Y., Landon, B., Willoughby, D. A. (1984) The effect of therapeutic agents on cartilage degradation in-vivo. J. Pharm. Pharmacol. 36: 709–710