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Studies on interleukin-1β induced glycosaminoglycan release from rat femoral head cartilage in-vitro

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Rheumatoid arthritis is characterized by synovial proliferation and cartilage degradation. Animal studies designed to mimic these effects have shown that rat femoral head (RFH) cartilage, implanted in association with a granuloma, loses glycosaminoglycan (GAG) (DeBrito et al 1987; Bottomley et al 1988). Original experiments by Dingle et al (1979) illustrated the importance of catabolin derived from the synovium in inducing GAG loss from bovine nasal septum (BNS) in-vitro. This was later identified as interleukin-1 (Saklatvala et al 1984). We describe here recombinant human interleukin-1 beta (rhIL1β)-induced GAG loss from RFH, a source of articulating hyaline cartilage of relevance to the in-vivo models described above.

RFH were dissected aseptically from 110–120 g male CFHB rats (Interfauna) and, using a laminar flow cabinet, washed and placed in 2 mL Dulbecco's modified Eagle's medium (DMEM, Gibco) with 25 mM HEPES and 4·5 g L^{-1} glucose supplemented with penicillin (10 units mL^{-1}), streptomycin (10 μg mL^{-1}), sodium pyruvate (1 mM), glutamine (1 mM) and foetal calf serum (5%). DMEM with 20 mM HEPES (Flow Laboratories) resulted in inadequate buffering and poor reproducibility. Cartilages were preincubated for 24 h at 37°C in an atmosphere of 5% CO_2/95% air, placed in fresh medium containing either drug vehicle, rhIL1 1β with drug vehicle, or rhIL 1β with drug, and incubated for a further 5 days. In those experiments where [^{3}H]glycine uptake was assessed cartilages were pulsed for 16 h before termination with 1 μCi mL^{-1} [^{3}H]glycine (13·4 Ci $mmol^{-1}$, Amersham) and washed three times in 10 mg mL^{-1} glycine. Both media and cartilages were papain digested before liquid scintillation counting and assay for GAG content (Farndale et al 1986, modified for use with a microplate reader). Results were expressed as d min^{-1} [^{3}H]glycine taken up per cartilage, and μg GAG released into the medium per mg wet weight of cartilage. The rhIL1β was produced by expression of the carboxyl terminal 153 amino acids of the 269 amino acid precursor in *Escherichia coli*. The product was purified to homogeneity as assessed by SDS-PAGE. Its activity in the lymphocyte activation factor assay was 100 pg $unit^{-1}$ and the endotoxin content was less than 1 ng mg^{-1} as determined by Limulus assay. Data was analysed by ANOVA and using weighted comparison of means (RS-Explore, BBN Software).

RhIL1β induced a dose-related release of GAG into the incubation medium from a basal release of 5·81 ± 0·77 reaching a maximum of 12·71 ± 1·15 μg mg^{-1} ($P<0·001$, n=6) at 100 ng mL^{-1} rhIL1β with a half maximal effect at approximately 10 ng mL^{-1}. [^{3}H]glycine uptake was reduced from 747 ± 36 to 420 ± 16 d min^{-1} ($P<0·001$, n=40) in the presence of 100 ng mL^{-1} rhIL1β.

In general, neither the tested non-steroidal anti-inflammatory drugs (NSAIDs) nor anti-inflammatory steroids had any significant effect on rhIL1β-induced GAG loss (see Table 1). Chloroquine elicited a dose-related inhibition of GAG loss which was concomittant with a further inhibition of [^{3}H]glycine incorporation over and above the action of rhIL1β. Chloroquine incubated with cartilage in the absence of rhIL1β reduced basal [^{3}H]glycine uptake similarly from 805·0 ± 35·0 to 145·8 ± 8·8 d min^{-1} ($P<0·001$ at 100 μM).

Table 1. The action of anti-inflammatory agents on RFH GAG loss induced by 100 ng mL^{-1} rhIL1β. Cartilages were incubated for 5 days in DMEM with 5% FCS and 25 mM HEPES, some pulsed with 1 μCi mL^{-1} [^{3}H]glycine and all assayed for GAG loss into the medium (Farndale et al 1986).

Drug	Concn (μM)	GAG (μg mg^{-1})	(n)	[^{3}H]glycine (d min^{-1})	(n)
Vehicle		5·2 ± 0·4	16	684 ± 25	5
rhIL1β		13·2 ± 0·8***	16	426 ± 27***	5
Indomethacin	10	10·8 ± 1·0	9	411 ± 23	5
	30	11·4 ± 0·8	15	394 ± 32	5
	100	13·2 ± 1·0	16	339 ± 19	5
Vehicle		5·5 ± 0·4	15	915 ± 56	5
rhIL1β		11·8 ± 0·6***	16	484 ± 49***	5
Tiaprofenic Acid	10	12·8 ± 1·4	15	391 ± 16	5
	30	11·6 ± 1·7	9	ND	
	100	13·2 ± 1·7	9	ND	
Vehicle		3·5 ± 0·2	5	915 ± 56	5
rhIL1β		9·0 ± 1·4***	5	484 ± 49***	5
Naproxen	10	8·7 ± 0·9	5	369 ± 31	5
	30	8·2 ± 0·6	5	447 ± 22	5
	100	9·1 ± 0·7	5	436 ± 22	5
Vehicle		4·9 ± 0·4	15	684 ± 25	5
rhIL1β		12·7 ± 0·8***	15	426 ± 27***	5
Dexamethasone	1	13·4 ± 1·2	15	456 ± 12	5
	3	14·3 ± 1·2	15	422 ± 15	5
	10	13·8 ± 0·8	15	425 ± 16	5
Vehicle		3·2 ± 0·5	10	1479 ± 87	5
rhIL1β		13·2 ± 1·1***	10	674 ± 34***	5
Prednisolone	3	14·8 ± 0·6	10	605 ± 55	5
	10	15·4 ± 0·8	10	548 ± 26	5
	30	16·2 ± 0·7	10	550 ± 49	5
Vehicle		4·5 ± 0·3	17	684 ± 25	5
rhIL1β		13·5 ± 0·9***	16	426 ± 27***	5
Hydrocortisone	10	12·8 ± 0·5	10	380 ± 15	5
	30	12·1 ± 0·8	15	383 ± 24	5
	100	15·2 ± 1·1	15	354 ± 18	5
Vehicle		5·1 ± 0·3	34	684 ± 25	5
rhIL1β		13·6 ± 0·6***	32	426 ± 27***	5
Chloroquine	10	11·7 ± 0·6	24	393 ± 20	5
	30	10·3 ± 0·4 +++	27	339 ± 11	5
	100	5·2 ± 0·3 +++	30	194 ± 24 +++	5

*** $P<0·001$ rhIL1β vs Vehicle. +++ $P<0·001$ drug vs rhIL1β. (ANOVA RS-Explore, BBN Software).

A recent report has indicated that RFH does not lose significant quantities of GAG in response to rhIL1α (Desa et al 1988). Incubation conditions are critical for the expression of rhIL1β activity. RFH does not respond to rhIL1β when incubated in RPMI 1640 or DMEM with 2 g L^{-1} glucose (data not shown). Using the units quoted by Desa et al (1988) we still find a significant reduction in RFH GAG content from 631 ± 15·2 to 433·2 ± 14·5 μg cartilage ($P<0·001$, n=65) in the presence of 100 ng mL^{-1} rhIL1β using DMEM with 4·5 g L^{-1} glucose as described earlier. We have not, however, investigated the effect of rhIL1α.

Under the appropriate conditions therefore, RFH will respond to rhIL1β with a release of GAG into the incubation medium accompanied by a reduction in protein synthesis.

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NSAIDs have little action on the effect of rhIL1β, similar to results reported using BNS (Rainsford 1985) with porcine catabolin. We did not find that indomethacin potentiated rhIL1β-induced GAG loss as reported by Desa et al (1988) for rhIL1α (1988), in addition to this neither had naproxen nor tiaprofenic acid any action. Chloroquine, and some analogues, reduce the action of interleukin-1 (Rainsford et al 1986), however, from our results it is clear that chloroquine has a direct action on the cartilage in the absence of rhIL1β. Steroids do not antagonize the action of catabolin on GAG loss from BNS, as well as rhIL1β on RFH, but appear to reduce synthesis of catabolic activity by the synovium (Steinberg et al 1979).

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Definitive IUPAC Recommendations

The following definitive recommendations on nomenclature, terminology, and symbolism have been published since January 1988:

1. Glossary of terms used in Photochemistry. Pure Appl. Chem. (1988) 60: 1055
2. Names for Hydrogen Atoms, Ions, and Groups, and for Reactions involving them. Pure Appl. Chem. (1988) 60: 1115
3. Presentation of Molecular Parameter Values for Infrared and Raman Intensity Measurements. Pure Appl. Chem. (1988) 60: 1385
4. Nomenclature of Glycoproteins, Glycopeptides, and Peptidoglycans. Pure Appl. Chem. (1988) 60: 1389
5. Nomenclature for Cyclic Organic Compounds with Contiguous Formal Double Bonds (the δ-Convention). Pure Appl. Chem. (1988) 60: 1395
6. Nomenclature, Symbols, Units and their Usage in Spectrochemical Analysis—VII: Molecular Absorption Spectroscopy, Ultraviolet and Visible (UV/VIS). Pure Appl. Chem. (1988) 60: 1449
7. Nomenclature, Symbols, Units and their Usage in Spectrochemical Analysis—X: Preparation of Materials for Analytical Atomic Spectroscopy and Other Related Techniques. Pure Appl. Chem. (1988) 60: 1461
8. Electrochemical Corrosion Nomenclature. Pure Appl. Chem. (1989) 61: 19
9. System for Symbolic Representation of Reaction Mechanisms. Pure Appl. Chem. (1989) 61: 23
10. Detailed Linear Representation of Reaction Mechanisms. Pure Appl. Chem. (1989) 61: 57
11. Definitions of Terms relating to Individual Macromolecules, their Assemblies, and Dilute Polymer Solutions. Pure Appl. Chem. (1989) 61: 211
12. A Classification of Linear Single-Strand Polymers. Pure Appl. Chem. (1989) 61: 243

Comments on these recommendations would be welcomed, addressed to the originating IUPAC Commission (for addresses see the appropriate issue of Pure Appl. Chem.), with copies to Dr Alan McNaught, Secretary, Joint Royal Society Royal Society of Chemistry Panel on Chemical Nomenclature, Thomas Graham House, Science Park, Milton Road, Cambridge, CB4 4WF, UK.