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Synthesis of arachidonate cyclo-oxygenase products by rheumatoid and nonrheumatoid synovial lining in nonproliferative organ culture

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SUMMARY Specimens of human rheumatoid and nonrheumatoid synovial lining were maintained in nonproliferative organ culture for 20 hours. The culture fluids were then assayed for prostaglandin E_2 (PGE_2), thromboxane B_2 (TXB_2), and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) by specific radioimmunoassay. The presence of each of these substances was confirmed by gas chromatography and mass spectrometry. Rheumatoid tissue produced significantly more of each cyclo-oxygenase product than nonrheumatoid tissue.

The initial step in the biosynthesis of prostaglandins from the unsaturated fatty acid precursor (for example, arachidonic acid) is the formation of unstable cyclic endoperoxides (PGG_2 and PGH_2) by the enzyme *fatty acid cyclo-oxygenase.* The endoperoxides can be converted to thromboxane A_2 (TXA_2), prostacyclin (PGI_2), or the 'primary' prostaglandins (PGE_2, PGD_2, and $PGF_{2\alpha}$) (review[1]).

It is now generally accepted that 'primary' prostaglandins, in particular PGE_2, are important inflammatory mediators (review[2]). These prostaglandins have been detected in human synovial fluids collected from inflamed joints, and concentrations are reduced after treatment with nonsteroid anti-inflammatory drugs.[3 4] Furthermore, tissue cultures of rheumatoid synovial lining produce prostaglandins.[5 6] More recently thromboxane B_2 and 6-keto-$PGF_{1\alpha}$ (the stable hydrolysis products of thromboxane A_2 and prostacyclin respectively) have been detected in inflammatory exudates from experimental animals.[7] Also, synovial effusions from rheumatoid joints have been found to contain thromboxane B_2 and 6-keto-$PGF_{1\alpha}$ in addition to the 'primary' prostaglandins.[8 9]

In the present study we have investigated the capacity of human synovial lining tissue to generate cyclo-oxygenase products. Synovial explants have been maintained in nonproliferative organ culture,[10] and tissue from rheumatoid and nonrheumatoid patients has been compared. Some of these results have been reported to the British Pharmacological Society and to the Heberden Society.[11 12]

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Materials and methods

TISSUE CULTURE

Biopsy specimens of human synovial lining were taken either at arthrotomy for internal derangement or at synovectomy. The nonrheumatoid specimens were taken from the knee, either from quiescent joints or from otherwise normal joints after recent mechanical trauma. The rheumatoid specimens were taken mainly from the knee during synovectomy from patients who had 'definite' or 'classical' disease according to the diagnostic criteria of the American Rheumatism Association.[13]

All specimens were removed in a bloodless field within 10 min of the application of the tourniquet and were transferred to the laboratory in a sterile container on a gauze moistened with Trowell's T-8 culture medium (Gibco). The tissue was cut into segments, each of approximately 4 × 4 mm planar surface and of the thickness of the membrane (not exceeding 4 mm). These were maintained individually in Trowell's nonproliferative adult organ maintenance culture at the relevant pH for the tissue.[14] For some segments the culture medium contained indomethacin (Wellcome) at 10^{-4} M. Explants were maintained under an atmosphere of 95% oxygen and 5% carbon dioxide at 37°C for 20 hours.

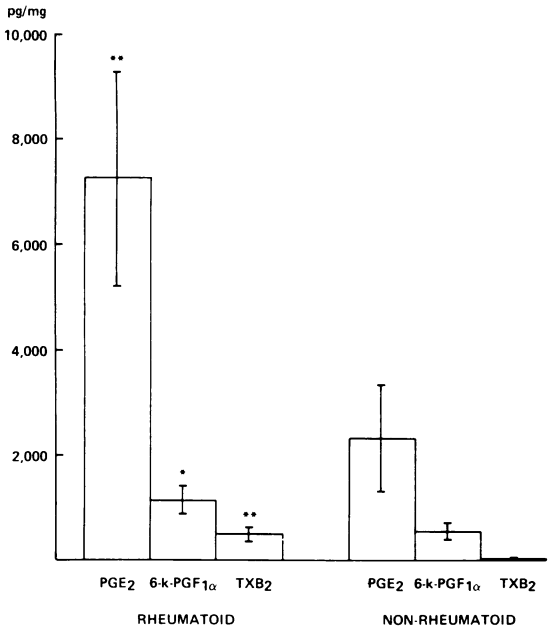

Fig. 1 *Mean concentrations of prostaglandin E_2 (PGE_2) 6-keto-prostaglandin $F_{1\alpha}$ (6-k-$PGF_{1\alpha}$) and thromboxane B_2 (TXB_2) in culture media from rheumatoid ($n=9$) and nonrheumatoid ($n=6$) synovial explants. The bars represent ±SE mean.*

$^*p<0{\cdot}1$, $^{**}p<0{\cdot}05$, compared with the nonrheumatoid group.

The medium was then withdrawn, snap-frozen to −70°C and stored at −20°C. After removal of excess water each specimen was weighed, then chilled by precipitate immersion in n-hexane (BDH 'low in aromatic hydrocarbons' grade, boiling range 67–70°C) at −70°C, and stored at −70°C in a corked dry glass tube. Histological confirmation of the pathology was obtained from suitably stained cryostat sections of the explants, prepared as described by Chayen *et al.*[15]

RADIOIMMUNOASSAY

PGE_2, 6-keto-$PGF_{1\alpha}$, and TXB_2 in diluted culture media were assayed directly by radioimmunoassays. These assays are relatively specific,[16] though PGE_2 does cross-react significantly (approximately 5%) in the assay for 6-keto-$PGF_{1\alpha}$. Therefore the cyclo-oxygenase products in several samples were extracted and separated by thin-layer chromatography prior to radioimmunoassay[7] to confirm data obtained by direct assay. The amount of each cyclo-oxygenase product generated in the culture period was expressed as pg/mg wet weight of tissue.

GAS-LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY

Cyclo-oxygenase products from 3 culture fluids were determined by gas liquid chromatography—mass spectrometry. Approximately 500 ng of each of the following internal standards were added to each fluid; 3,3,4,4-D_4-6-keto-$PGF_{1\alpha}$, 5,6,8,11,12,14,15-D_7-PGE_2, and 5,6,8,9,11,12,14,15-D_8-TXB_2. The fluids were mixed with 2 volumes of ice-cold acetone to precipitate protein, and then the supernatant was washed with 2 volumes of n-hexane to remove neutral lipids. The remaining aqueous-acetone phase was acidified to pH 4 with citric acid and extracted twice with 2 volumes of chloroform. The combined extracts were evaporated to dryness and the residues were subjected to silicic acid column chromatography with mixtures of chloroform and methanol to elute the cyclo-oxygenase products.[17] Fraction II, which contains PGE_2, TXB_2, and 6-keto-$PGF_{1\alpha}$, was concentrated to dryness and then reacted successively with methoxamine hydrochloride (Pierce Chemical Co.) diazomethane and N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS; Pierce Chemical Co.) as previously described.[7] Aliquots of the derivatised samples and standards were injected into a Hewlett-Packard Model 5730 A GLC combined with a VG Micromass 16F mass spectrometer; a 1% OV1 column was employed at 230°C. The following ions were monitored: m/z 301·20 (TXB_2), 305·23 (D_8-TXB_2), 508·33 (PGE_2 and 6-keto-$PGF_{1\alpha}$), 512·35 (D_4-6-keto-$PGF_{1\alpha}$), and 515·37 (D_7-PGE_2). Under the chromatography conditions employed the retention times of the derivatives of PGE_2, TXB_2, and 6-keto-$PGF_{1\alpha}$ were 5m 58s, 6m 53s, and 7m 32s respectively.

Results

The results presented in Tables 1 and 2 and Fig. 1 are derived from radioimmunoassay of culture media directly. Culture fluids from samples 2207, 2208 (Table 1), and 2209 (Table 2) were extracted and purified before radioimmunoassay, and these experiments confirmed both the qualitative and quantitative results obtained by direct assay. The presence of PGE_2, 6-keto-$PGF_{1\alpha}$, and TXB_2 in extracts of culture fluids from sample 2342 (Table 2) was positively confirmed by gas-liquid chromatography and mass spectrometry.

All the culture fluids tested contained detectable concentrations of PGE_2 and 6-keto-$PGF_{1\alpha}$, and in each case, with the exception of sample 2207 (Table 1), PGE_2 was the predominant cyclo-oxygenase product. All the rheumatoid tissues produced significant amounts of TXB_2, but in most cases it was the minor

Table 1 *Generation of cyclo-oxygenase products by nonrheumatoid synovial explants during 20 h nonproliferative culture*

Patient	*Sex*	*Age*	*Diagnosis*	*Joint*	*Treatment*	*pg/mg wet wt. Synovial tissue*		
						PGE_2	6-k-$PGF_{1\alpha}$	TXB_2
2207	F	73	Torn degenerated medial cartilage	Knee	None	27	40	0
2208	F	13	Chondromalacia	Knee	None	2174	856	0
2267	M	31	Chondromalacia (diabetic)	Knee	Insulin	7047	1017	105
2270	M	43	Torn meniscus	Knee	None	936	690	17
2316	M	19	Torn meniscus	Knee	None	2551	256	40
2358	F	16	Chondromalacia	Knee	None	1188	607	31

Table 2 *Generation of cyclo-oxygenase products by rheumatoid synovial explants during 20 h nonproliferative culture*

Patient	*Sex*	*Age*	*Diagnosis*	*Joint*	*Treatment*	*pg/mg wet wt. Synovial tissue*		
						PGE_2	6-k-PGF	TXB_2
2209	F	41	Rheumatoid arthritis	Knee	Gold	1143	809	709
2273	M	43	" "	Knee	Distalgesic, Indocid, Fenopron, Clinoril	1998	276	140
2311	F	53	" "	Knee	Froben, paracetamol	5131	974	29
2328	F	63	" "	Knee	Benoral, imipramine, gold	4725	2464	1103
2334	F	55	" "	Knee	Indocid, Orudis	7742	1093	726
2342	F	59	" "	Knee	Ponstan, Depo-medrone	12924	2374	398
2353	M	22	" "	Knee	Diclofenac, nitrazepam	4196	838	175
2393	F	51	" "	Knee	Penicillamine, Distalgesic, Dolobid	20900	583	834
2439	F	55	" "	Wrist	Indocid	6553	765	183

Proprietary names: Distalgesic (dextropropoxyphene), Indocid (indomethacin), Fenopron (fenoprofen), Clinoril (sulindac), Froben (flurbiprofen), Benoral (benorylate), Orudis (ketoprofen), Ponstan (mefenamic acid), Depo-medrone (methyl prednisolone), Dolobid (diflunisal).

product (Table 2). In 2 out of 6 cultures of nonrheumatoid tissues TXB_2 was not detected (Table 1). The mean production of each cyclo-oxygenase product by rheumatoid tissue was significantly higher than mean values for the nonrheumatoid group (Fig. 1). Indomethacin (10^{-5} to 10^{-3} M) caused a dose-dependent inhibition of the generation of each cyclo-oxygenase product and in tissues from 7 different patients 10^{-4} M indomethacin suppressed cyclo-oxygenase activity by at least 85%.

Discussion

The results presented in this paper confirm the observations that synovial lining explants produce PGE_2[5,6] and support the findings that rheumatoid synovium has a greater prostaglandin synthetase capacity than nonrheumatoid synovium.[18,19] They also show that these tissues are capable of generating thromboxanes and prostacyclin, though PGE_2 is the predominant product in both groups. It is possible therefore that the TXB_2 and 6-keto-$PGF_{1\alpha}$ detected in synovial fluids[8,9] originate from synovial tissue.

Prostacyclin, like PGE_2, is a potent vasodilator and hyperalgesic agent,[2] and generation of prostacyclin as well as PGE_2 by inflamed tissues could contribute to inflammatory symptoms such as erythema, oedema, and pain. The role of thromboxanes in inflammation is less clear. Thromboxane A_2 is a potent vasoconstrictor and aggregator of platelets,[1] and these properties may reduce haemorrhage at an inflamed site. Also, thromboxane B_2 has been reported to have chemotactic activity for leucocytes.[20] There are, however, no reports as yet which indicate that thromboxanes are important inflammatory mediators. The increased cyclo-oxygenase activity in rheumatoid tissue may be fundamental to the rheumatoid process, or it may be a less specific consequence of the pathology of these joints.

Generation of cyclo-oxygenase products by synovial tissue in culture appears to be independent of prior drug treatment. Tissues from patients receiving nonsteroid anti-inflammatory drugs such as indomethacin, flurbiprofen, or diclofenac, which are known to inhibit prostaglandin synthesis,[2] do not have significantly less cyclo-oxygenase activity than tissues from patients receiving other drugs (Table 2). These observations are in agreement with the findings that preparations of human synovial microsomes from patients receiving indomethacin, naproxen, or ibuprofen did not have decreased cyclo-oxygenase activity.[21] These authors reported, however, that tissues from patients taking aspirin did not generate prostaglandins in vitro, and they attributed this to an irreversible effect of aspirin which was not shared by other nonsteroid anti-inflammatory drugs.

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References

1 Moncada S, Vane J R. Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A_2 and prostacyclin. *Pharmacol Rev* 1979; **30:** 293–331.

2 Higgs G A, Moncada S, Vane J R. The mode of action of anti-inflammatory drugs which prevent the peroxidation of arachidonic acid. *Clin Rheum Dis* 1980; **61:** 675–93.

3 Higgs G A, Vane J R, Hart F D, Wojtulewski J A. Effects of anti-inflammatory drugs on prostaglandins in rheumatoid arthritis. In: Robinson H J, Vane J R, eds. *Prostaglandin Synthetase Inhibitors.* New York: Raven Press, 1974: 165–73.

4 Robinson D R, Levine L. Prostaglandin concentrations in synovial fluid in rheumatic diseases: action of indomethacin and aspirin. In: Robinson H J, Vane J R, eds. *Prostaglandin Synthetase Inhibitors.* New York: Raven Press, 1974: 223–8.

5 Robinson D R, Smith H, McGuire M B, Levine L. Prostaglandin synthesis by rheumatoid synovium and its stimulation by colchicine. *Prostaglandins* 1975; **10:** 67–85.

6 Sturge R A, Yates D B, Gordon D, Franco M, Paul W, Bray M, Morley J. Prostaglandin production in arthritis. *Ann Rheum Dis* 1978; **37:** 315–20.

7 Higgs G A, Salmon J A. Cyclo-oxygenase products in carrageenin-induced inflammation. *Prostaglandins* 1979; **17:** 737–46.

8 Trang L E, Granstrom E, Lovgren O. Levels of prostaglandins $F_{2\alpha}$ and E_2 and thromboxane B_2 in joint fluid in rheumatoid arthritis. *Scand J Rheumatol* 1977; **6:** 151–4.

9 Brodie M J, Hensby C N, Parke A, Gordon D. Is prostacyclin the major pro-inflammatory prostanoid in joint fluid? *Life Sci* 1980; **27:** 603–8.

10 Trowell O A. The culture of mature organs in synthetic medium. *Exp Cell Res* 1959; **16:** 118–47.

11 Bitensky L, Cashman B, Chayen J, Henderson B, Higgs G A, Salmon J A, Vane J R. Synthesis of arachidonate cyclo-oxygenase products by rheumatoid and non-rheumatoid synovial tissue. *Br J Pharmacol* 1981; **72:** 522P–523P.

12 Higgs G A, Salmon J A, Cashman B, Henderson B, Bitensky L, Chayen J, Vane J R. Products of cyclo-oxygenase activity from human synovium. *Ann Rheum Dis* 1981; **40:** 190.

13 Ropes M W, Bennet G A, Cobb S, Jessar R A. Diagnostic criteria for rheumatoid arthritis. *Ann Rheum Dis* 1951; **18:** 49.

14 Poulter L W, Bitensky L, Cashman B, Chayen J. The maintenance of human synovial tissue *in vitro. Virchows Arch (Cell Pathol)* 1970; **4:** 303–9.

15 Chayen J, Bitensky L, Butcher R G. *Practical Histochemistry.* New York, London: Wiley, 1973.

16 Salmon J A. A radioimmunoassay for 6-keto-prostaglandin $F_{1\alpha}$. *Prostaglandins* 1978; **15:** 383–97.

17 Salmon J A, Karim S M M. Methods for analysis of prostaglandins. In: Karim S M M, ed. *Prostaglandins: Chemical and Biochemical Aspects.* Lancaster: MTP. 1976: 25–85.

18 Chaintreuil J, Blotman F, Flandre O, Crastes de Paulet A, Simon L. Prostaglandin biosynthesis by normal and rheumatoid human synovial tissue: A comparative study on microsomes and whole homogenates. *Eur J Rheumatol* 1979; **2:** 119–21.

19 Blotman F, Chaintreuil J, Poubelle P, Flandre O, Crastes de Paulet A, Simon L. PGE_2, $PGF_{2\alpha}$ and TXB_2 biosynthesis by human rheumatoid synovia. In: Samuelsson B, Ramwell P W, Paoletti R, eds. *Advances in Prostaglandin and Thromboxane Research.* New York: Raven Press, 1980: **8:** 1705–8.

20 Kitchen E A, Boot J R, Dawson W. Chemotactic activity of thromboxane B_2, prostaglandins and their metabolites for polymorphonuclear leukocytes. *Prostaglandins,* 1978; **16:** 239–44.

21 Crook D, Collins A J, Bacon P A, Chan R. Prostaglandin synthetase activity from human rheumatoid synovial microsomes. Effect of 'aspirin-like' drug therapy. *Ann Rheum Dis* 1976; **35:** 327–32.