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THE SYNOVIAL PROSTAGLANDIN SYSTEM IN CHRONIC INFLAMMATORY ARTHRITIS: DIFFERENTIAL EFFECTS OF STEROIDAL AND NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

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1 The present study was undertaken to characterize the spectrum of arachidonic acid metabolites present in synovial effusions of patients with rheumatoid or psoriatic arthritis, and to compare changes in their concentration following a short-term treatment with 6α-methyl-prednisolone (6-MeP: 4–8 mg/day) or indoprofen (1.2 g/day), a nonsteroidal anti-inflammatory agent with proven synovial prostaglandin inhibitory effect.

2 Measurements of prostaglandin E_2 (PGE_2), thromboxane (TX) B_2, 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ were performed by radioimmunoassay techniques in synovial effusions obtained from 23 patients, and validated by thin-layer chromatographic analysis of the extracted immunoreactivity.

3 PGE_2 and TXB_2 accounted for more than 60% of the total immunoreactivity in untreated patients. The absence of any constant ratio between the different arachidonic acid metabolites detected in synovial fluid is consistent with a heterogeneous cellular origin of these compounds.

4 Indoprofen treatment was associated with a consistent reduction of synovial prostaglandin and thromboxane concentrations, ranging from 36% in the case of 6-keto-$PGF_{1\alpha}$ to 90% in the case of PGE_2.

5 In contrast, 6-MeP caused opposite changes on different metabolites originating via the cyclo-oxygenase pathway. Thus, 6-keto-$PGF_{1\alpha}$ concentrations were reduced by 35%, $PGF_{2\alpha}$ concentrations were increased by 30%, while PGE_2 and TXB_2 were unchanged following 6-MeP.

6 Although the mechanism(s) underlying the failure of 6-MeP to reduce synovial PGE_2 and TXB_2 levels are uncertain, the results of the present study clearly indicate that therapeutic doses of steroidal and nonsteroidal anti-inflammatory drugs cause quite distinct changes in arachidonic acid metabolism, which might be relevant to their specific therapeutic actions and side-effects.

Introduction

The discovery by Vane and his colleagues that aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) inhibited prostaglandin synthesis and release (Vane, 1971; Ferreira, Moncada & Vane, 1971; Smith & Willis, 1971) has raised considerable interest in the role of this family of compounds in the pathogenesis of chronic inflammatory arthropathies. Detectable prostaglandin levels have been reported in synovial effusions from patients with rheumatoid arthritis (Levine, 1973; Higgs, Vane, Hart & Wojtulewski, 1974; Patrono, Grossi-Belloni, Serra, Bombardieri, Mancuso & Gorga, 1974; Swinson, Bennett & Hamilton, 1976; Trang, Granström & Lövgren, 1977; Sturge, Yates, Gordon, Franco, Paul, Bray & Morley, 1978), with consistent evidence of their suppression in patients undergoing treatment with NSAIDs. Moreover, anti-inflammatory steroids have been reported to inhibit prostaglandin biosynthesis by preventing the release and/or the activation of phospholipase(s), the enzyme(s) which releases arachidonic acid from membrane phospholipids and therefore supplies the substrate for prostaglandin synthesis (Flower & Blackwell, 1976). However, very limited information is available on the *in vivo* effects of steroids on synovial fluid prostaglandin levels (Higgs *et al.*, 1974; Patrono *et al.*, 1974). Since different cell populations can be expected to give a qualitatively as well as quantitatively variable contribution to the measured synovial prostaglandin levels and to be variably affected by pharmacological treatment, the present study was undertaken to characterize the spectrum of arachidonic acid metabolites present in synovial effusions and to compare changes in their concentration following a short-term steroidal or nonsteroidal treatment in patients with rheumatoid or psoriatic arthritis.

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Methods

Patients

Patients with either adult or juvenile (J) rheumatoid arthritis (RA) or psoriatic arthritis seen at the Rheumatic Disease Unit of the University of Pisa were admitted to this study which was performed with the approval of the Ethical Committee. They were selected on the basis of the following criteria: (1) presence of at least one large joint effusion; (2) spontaneous interruption (usually for fear of side effects or reputed inefficacy) of any steroidal, as well as nonsteroidal anti-inflammatory therapy; (3) absence of any 'basic' treatment (gold, anti-malarials, penicillamine, immunosuppressors) for at least 6 months. Between March 1976 and March 1980, 23 patients fulfilled these criteria: they were 17 patients with classical or definite RA (Ropes, Bennett, Cobb, Jacox & Jessar, 1958), 4 with JRA and 2 with psoriatic arthritis. Informed written consent was obtained from each patient and/or his/her parents.

Study protocol

Patients selected were hospitalized and left untreated for at least 7 days; a synovial fluid specimen was then aspirated from the same knee joint immediately before and after 4–8 days of treatment with either 6α-methyl-prednisolone (6-MeP: 4–8 mg/day at 08 h 00 min), or indoprofen (1.2 g/day in 4 divided doses) a recently described nonsteroidal anti-inflammatory agent with proven synovial prostaglandin inhibitory effect in patients with rheumatoid arthritis (Caruso, Moro, Patrono, Sacchetti, Tamassia & Tosolini, 1980). Joint fluid was always drawn at the same time of the day (approx. 11 h 00 min). Synovial fluid specimens were examined for protein content, leucocyte count, haemolytic complement level (CH50) and prostaglandin levels. Paired contemporary blood samples were tested for erythrocyte sedimentation rate (ESR: Westergreen), leucocyte count, protein content and CH50 levels.

Collection of synovial fluid

Synovial fluids for prostaglandin measurement were aspirated from the lateral aspect of the knee with a 0.7 mm needle, without anaesthesia and with a fenoprofen-sodium solution (final concentration 10 μg/ml) in the syringe. Samples were immediately centrifuged at 700 *g* at 4°C for 15 min, and the supernatant stored at −20°C until used. Other aliquots of the same synovial fluid were collected separately and tested for leucocyte count, protein content, latex test and CH50 levels.

Complement titration

CH50 levels were measured in both serum and synovial fluid by the method of Kent & Fife (1963). Paired sera and synovial fluids were measured in the same assay.

Protein measurement

Protein levels in sera and synovial fluids were measured by the biuret method.

Prostaglandin and thromboxane measurements

Synovial fluids were analysed for PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ (the stable breakdown product of PGI_2) and TXB_2 (the stable breakdown product of TXA_2) by radioimmunoassay (RIA). Aliquots (150 μl) of the unextracted samples were assayed in triplicate at a 1:10 dilution in the standard diluent of the assay, as previously described (Patrono, Bombardieri, Di Munno, Pasero, Greco, Grossi-Belloni & Ciabattoni, 1976). The details of binding affinity and immunological specificity of anti-$PGF_{2\alpha}$ (GP 705) and anti-PGE_2 (GP 356) sera are described elsewhere (Ciabattoni, Pugliese, Spaldi, Cinotti & Patrono, 1979). Using 4,000–5,000 d/min of [^{3}H]-PGE_2 or [^{3}H]-$PGF_{2\alpha}$ (Amersham Radiochemical Centre: 160–180 Ci/mmol) in a volume of 1.5 ml, the lowest detectable concentration of both compounds was 10 pg/ml of synovial fluid. TXB_2 concentrations were measured by a recently described RIA method (Patrono, Ciabattoni, Pugliese, Pinca, Castrucci, De Salvo, Satta & Parachini, 1980).

Using 4,000–5,000 d/min of [^{3}H]-TXB_2 (New England Nuclear: 150 Ci/mmol) and anti-TXB_2 serum diluted 1:750,000 in a volume of 1.5 ml, the lowest detectable concentration was 5 pg/ml of synovial fluid. The 6-keto-$PGF_{1\alpha}$ RIA employed [^{3}H]-6-keto-$PGF_{1\alpha}$ (New England Nuclear: 100 Ci/mmol) and an anti-6-keto-$PGF_{1\alpha}$ serum kindly provided by Dr B.A. Peskar (University of Freiburg). The binding characteristics of this antiserum have been recently described (Ciabattoni, Pugliese, Cinotti & Patrono, 1980). Using 4,000–5,000 d/min of [^{3}H]-6-keto-$PGF_{1\alpha}$ and anti-6-keto-$PGF_{1\alpha}$ serum diluted 1:200,000 in a volume of 1.5 ml, the lowest detectable concentration was 10 pg/ml of synovial fluid. None of the drugs used interfered with these determinations.

Thin layer chromatography

The nature of prostaglandin and TXB_2-like immunoreactivity detected in synovial fluid extracts was characterized by means of thin layer chromatography

Table 1 Main clinical data and serological changes after treatment in 23 patients with chronic inflammatory arthritis

Patient	*Age/sex*	*Diagnosis*	*Treatment duration* (days)	*Treatment dosage* ($mg\,kg^{-1}\,day^{-1}$)	*ESR* (mm/1 h) b	a	*WBC* ($10^3/mm^3$) b	a	*Proteins* (g/dl) b	a	*CH50* (u/ml) b	a
6α-Methyl-prednisolone-treated group												
1	28 M	RA	4	0.057	16	10	4.9	4.9	7.9	8.1	375	250
2	38 M	RA	5	0.086	138	135	—	4.3	7.9	8.4	271	350
3	67 F	RA	4	0.080	50	68	3.4	3.4	7.6	7.3	285	350
4	29 M	PA	7	0.061	25	10	4.3	8.2	—	6.9	296	—
5	73 M	RA	7	0.057	75	20	—	9.3	7.2	—	335	—
6	50 F	RA	5	0.067	36	38	7.2	8.0	6.8	6.9	350	300
7	46 F	RA	5	0.107	54	60	3.7	5.0	6.8	6.7	296	—
8	59 M	RA	8	0.053	24	—	6.1	—	—	6.7	222	242
9	14 M	JRA	4	0.060	116	93	4.7	4.7	7.3	7.2	168	188
10	21 M	PA	7	0.056	100	55	8.7	—	6.9	—	335	—
11	50 F	RA	7	0.055	35	—	6.4	—	6.9	5.9	266	264
12	65 F	RA	6	0.100	41	50	7.0	9.6	6.2	6.4	228	236
13	13 F	JRA	7	0.086	17	—	3.9	—	6.6	5.7	252	197
14	22 F	RA	7	0.102	9	6	12.5	9.2	7.4	7.2	222	200
15	17 M	JRA	5	0.062	24	26	5.2	8.8	6.7	6.3	268	273
16	23 M	RA	5	0.127	21	16	6.1	6.4	7.4	7.0	326	275
17	58 M	RA	5	0.154	112	94	5.0	6.8	6.8	6.8	193	206
Indoprofen-treated group												
18	54 F	RA	5	20	75	63	3.9	3.3	—	6.2	155	—
19	45 F	RA	4	19	97	110	5.6	—	5.6	—	350	280
20	42 M	RA	6	17	80	42	7.5	6.8	7.3	7.4	300	—
21	14 M	JRA	5	18	119	95	7.3	6.7	7.3	6.8	236	326
22	53 M	RA	6	17	110	70	7.2	6.8	8.0	7.6	318	337
23	50 F	RA	4	19	100	77	4.4	4.2	7.2	7.2	322	377

b = before; a = after; RA = rheumatoid arthritis; PA = psoriatic arthritis; JRA = juvenile rheumatoid arthritis.

(t.l.c.), as previously described for urinary extracts (Ciabattoni *et al.*, 1979).

Two pools of 'high' (> 150 pg/ml) and 'low' (< 30 pg/ml) PGE_2 content were prepared by mixing 2–3 ml of 8 synovial fluid samples each. Approx. 4,000 ct/min of [^{3}H]-$PGF_{2\alpha}$ in 2 ml of 0.02 M PO_4 buffer, pH 7.4, were added to 20 ml of synovial fluid to serve as a tracer during extraction and purification. The choice of $PGF_{2\alpha}$ was based on verification of its extraction and elution from silica gel with the same efficiency as PGE_2, TXB_2 and 6-keto-$PGF_{1\alpha}$. Synovial fluid was acidified to pH 3.0 and extracted with 3 volumes of cyclohexane: ethyl-acetate 1:1 (v/v). The solvent was removed by evaporation at 18°C. The extracted residue was dissolved in 0.5 ml methanol and dried down to 0.1 ml. After counting a 0.01 ml aliquot for recovery, the remainder was applied to a commercial glass plate (Silica Gel 60F-254, Merck) and co-chromatographed with authentic prostaglandins and TXB_2. After developing the plate, the separated immunoreactivity was recovered and assayed for PGE_2, $PGF_{2\alpha}$, TXB_2 and 6-keto-$PGF_{1\alpha}$, as described elsewhere in detail (Ciabattoni *et al.*, 1979).

Drugs

The drugs used were: fenoprofen sodium (Eli Lilly and Co.), indoprofen (Carlo Erba), 6α-methylprednisolone (Hoechst).

Statistical analysis

Statistical analysis of the data was performed using the paired Student's *t* test.

Results

A total of 17 patients with chronic inflammatory arthritis received 6-MeP (0.08 ± 0.03 mg/kg, mean ± s.d.) for an average of 5.8 ± 1.3 days (range 4–8 days), while 6 patients were treated with indoprofen (18.3 ± 1.2 mg/kg, mean ± s.d.) for 5.0 ± 0.9 days (range 4–6 days). The individual clinical data as well as serological changes induced by both therapeutic regimens are given in Table 1. The measured synovial parameters are shown in Table 2. Neither treatment caused any statistically significant change in leucocytes. Differential leucocyte counts (not shown) were similarly not affected to any significant extent. However, marked differences were noted between steroidal and nonsteroidal treatment on the other parameters. Thus, 6-MeP caused a statistically significant decrease of proteins and CH50 levels, while these were not affected by indoprofen.

Before treatment, measurable concentrations of PGE_2, $PGF_{2\alpha}$, TXB_2 and 6-keto-$PGF_{1\alpha}$ were present in most synovial fluids. Although PGE_2 and TXB_2 accounted for > 50% of the measured immunoreactivity in the vast majority of patients, no consistent ratio was found among different cyclo-oxygenase-derived products. Prostaglandin and TXB_2-like immunoreactivity present in synovial extracts was eluted from t.l.c. plates with an overall recovery of 60–70%. Figure 1 depicts the pattern of t.l.c. distribution of PGE_2-like immunoreactivity present in 2 pools of synovial fluids with 'high' and 'low' concentration. In both instances, the immunoreactivity detected in the methanol eluates of silica gel segments of the plate showed an identical chromatographic behaviour to authentic PGE_2. Qualitatively similar results (not shown) were obtained in terms of

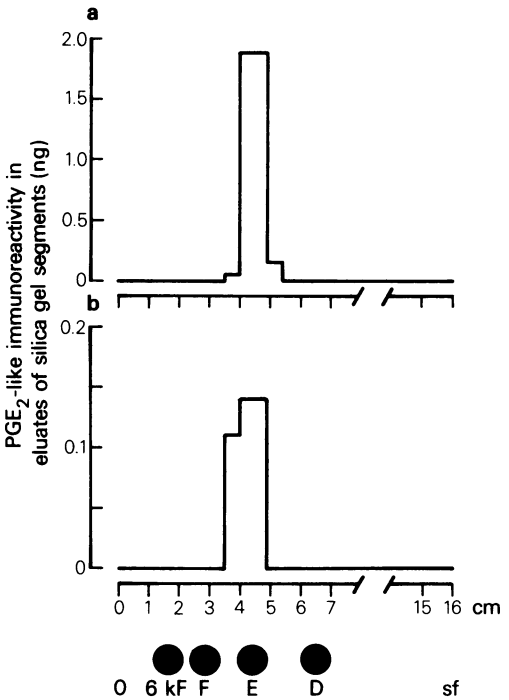

Figure 1 Chromatographic behaviour of synovial prostaglandin E_2 (PGE_2)-like immunoreactivity detected in two pools with 'high' (a) and 'low' (b) PGE_2 concentrations. Synovial extracts were subjected to t.l.c. in the organic phase of ethyl acetate, iso-octane, acetic acid, water (11:5:2:10). After development, each lane of the plate was divided into 1 cm segments, the silica gel scraped off and eluted with methanol. All the eluates were assayed for PGE_2-like immunoreactivity by anti-PGE_2 serum GP 356. The total amount of immunoreactivity detected in each segment is plotted. The dots under the abscissa scale indicate the location of co-chromatographed authentic prostaglandins: O, origin, 6kF, 6-keto-$PGF_{1\alpha}$; F, $PGF_{2\alpha}$; E, PGE_2; D, PGD_2; sf, solvent front.

Table 2 Synovial fluid parameters of 23 patients with inflammatory arthritis before (b) and after (a) anti-inflammatory treatment

	Leucocytes (10^3/mm^3)		*Proteins* (g/dl)		*CH50* (u/ml)		*PGE_2* (pg/ml)		*$PGF_{2\alpha}$* (pg/ml)		*TXB_2* (pg/ml)		*6-keto-$PGF_{1\alpha}$* (pg/ml)	
Patient	b	a	b	a	b	a	b	a	b	a	b	a	b	a
6α-Methyl-prednisolone-treated group														
1	7.9	6.7	6.3	6.4	208	161	66	86	28	46	105	68	15	0
2	22.8	2.5	—	5.5	192	—	180	20	47	20	—	90	—	—
3	8.2	7.2	4.1	4.3	50	50	30	50	20	35	90	52	100	28
4	12.3	10.4	5.2	4.7	122	85	52	54	20	20	135	150	200	90
5	18.3	20.7	4.7	4.3	111	57	50	50	35	35	300	105	200	200
6	24.0	28.0	4.6	4.0	160	117	31	28	43	31	—	480	—	—
7a	14.0	21.9	4.8	4.7	92	65	125	290	32	31	98	105	200	70
7b	11.5	18.7	4.4	4.5	78	77	94	110	20	22	99	105	182	180
8	19.0	4.2	—	3.4	73	50	30	28	20	43	—	—	—	—
9	5.0	4.8	5.1	5.0	98	87	25	35	20	30	—	180	230	120
10	75.2	48.0	4.8	4.5	236	210	280	120	30	35	60	50	320	220
11	3.8	3.5	4.2	4.0	58	76	95	105	14	34	60	0	0	0
12	35.5	31.0	8.4	8.8	50	50	130	166	93	155	308	480	92	91
13	11.4	13.7	4.4	4.2	50	50	49	90	11	19	87	52	0	0
14	10.2	15.0	5.1	5.3	117	67	20	42	20	33	60	68	0	0
15	15.5	12.8	4.2	4.1	76	83	250	430	33	55	293	267	0	5
16	5.3	2.4	4.5	4.2	167	136	105	110	20	33	52	38	10	0
17	11.0	9.6	4.8	4.3	50	50	290	130	50	30	145	187	0	0
Mean	17.3	14.5	5.0	4.8*	106	81****	106	108	31	39*	135	123	103	67**
±s.e.mean	±3.9	±2.8	±0.3	±0.3	±14	±11	±21	±24	±4	±7	±25	±33	±28	±21
Indoprofen-treated group														
18a	11.4	11.0	—	—	50	50	55	20	20	20	297	52	170	85
18b	11.4	6.2	—	—	50	50	130	20	26	20	125	60	—	—
19	16.0	11.2	4.0	4.3	64	50	220	13	45	16	225	169	410	320
20	17.5	12.3	5.5	4.4	230	156	76	20	20	20	187	90	230	120
21a	14.8	27.7	5.3	5.5	186	—	240	0	24	10.5	172	120	280	190
21b	40.1	31.0	5.4	5.7	192	—	200	12	24	12	188	127	320	200
22	15.0	15.6	5.4	4.5	90	159	85	35	14	0	75	40	130	70
23	11.0	7.8	5.1	5.3	50	50	160	0	32	0	142	0	10	0
Mean	17.1	15.4	5.1	5.0	89	86	146	15****	26	12**	176	82***	221	141****
±s.e.mean	±3.4	±3.2	±0.2	±0.3	±29	±23	±25	±4	±3	±3	±24	±19	±50	±40

*$P<0.05$; **$P<0.01$; ***$P<0.005$; ****$P<0.0025$, paired Student's t test.

$PGF_{2\alpha}$-, TXB_2- and 6-keto-$PGF_{1\alpha}$-like immunoreactivities by assaying the same eluates with anti-$PGF_{2\alpha}$, anti-TXB_2 and anti-6-keto-$PGF_{1\alpha}$ sera, respectively. Failure of these antisera to detect any appreciable amount of immunoreactivity in areas other than those corresponding to the homologous compounds strongly supports the identification of the synovial immunoreactive components as PGE_2, $PGF_{2\alpha}$, TXB_2 and 6-keto-$PGF_{1\alpha}$.

As shown in Figure 2, 6-MeP had opposite effects on different arachidonic acid metabolites detected in synovial fluid. In contrast, indoprofen treatment was associated with a consistent though variable reduction of all compounds measured, ranging from 36% in the case of 6-keto-$PGF_{1\alpha}$ to 90% in the case of PGE_2.

When examining individual values, it becomes apparent that variable responses to 6-MeP were obtained in different patients. Thus, 8 of 18 (44%) synovial fluid samples showed an increase of PGE_2 concentrations following 6-MeP, while 3/18 (17%) displayed a decrease and 7/18 (39%) no change. In contrast, 8/8 samples showed a quite dramatic decrease of PGE_2 concentrations following indoprofen treatment. A similar pattern was noted in terms of $PGF_{2\alpha}$ concentrations: 10/18 samples (55%) showed an increase, 3/18 (17%) a decrease and 5/18 (28%) no change following 6-MeP. Six out of 8 samples obtained from indoprofen-treated patients showed an appreciable decrease of $PGF_{2\alpha}$ concentrations. In 2/14 (14%) samples, TXB_2 concentrations were increased following 6-MeP, while 6/14 (43%) showed a decrease and 6/14 (43%) no change. All samples obtained following indoprofen treatment showed a substantial drop of TXB_2 levels. In 5/15 (33%) samples, 6-keto-$PGF_{1\alpha}$ concentrations were decreased following 6-MeP, while 10/15 (67%) showed no substantial change. In contrast, indoprofen caused an appreciable decrease of 6-keto-$PGF_{1\alpha}$ concentrations in all samples.

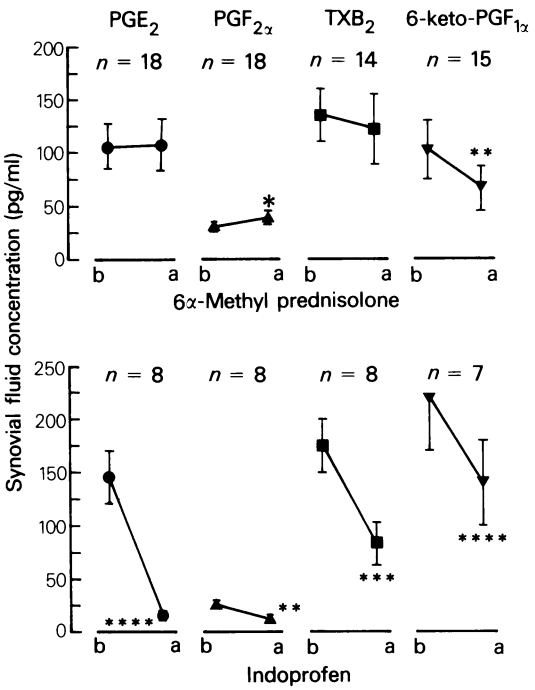

Figure 2 Synovial fluid concentrations of prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$, thromboxane B_2 (TXB_2) and 6-keto-$PGF_{1\alpha}$ in patients with rheumatoid or psoriatic arthritis, before (b) and after (a) a short-term treatment with 6α-methyl prednisolone (upper panel) or indoprofen (lower panel). Mean values are represented; vertical lines show s.e.mean. The numbers refer to the number of samples. *P* value refers to significance of differences between drug treatment and control: $*P<0.05$; $**P<0.01$; $***P<0.005$; $****P<0.0025$, paired Student's *t* test.

Discussion

Arachidonic acid metabolism generates a variety of biologically active products, which may be involved in some aspects of acute as well as chronic inflammation (for review see Higgs, Moncada & Vane, 1979). These substances include cyclo-oxygenase derivatives, i.e. the classical prostaglandins (PGE_2, $PGF_{2\alpha}$, PGD_2), TXA_2 and PGI_2 as well as lipoxygenase-derived products, i.e. hydroxy acids and the recently discovered leukotrienes (Samuelsson, Borgeat, Hammarström & Murphy, 1979).

In the present study, we have characterized the spectrum of cyclo-oxygenase-derived products of arachidonic acid metabolism present in the synovial fluid of patients with chronic inflammatory arthritis. Our results confirm earlier reports of several investigators (Levine, 1973; Higgs *et al.*, 1974; Patrono *et al.*, 1974; Swinson *et al.*, 1976; Trang *et al.*, 1977; Sturge *et al.*, 1978) that synovial fluid effusions from patients with rheumatoid arthritis contain detectable amounts of PGE_2, $PGF_{2\alpha}$ and TXB_2; but, in addition, demonstrate the presence of comparable concentrations of 6-keto-$PGF_{1\alpha}$, the stable breakdown product of PGI_2. The presence of 6-keto-$PGF_{1\alpha}$ in carrageenin-induced inflammatory exudates has been described recently (Higgs & Salmon, 1979). PGE_2, TXB_2 and 6-keto-$PGF_{1\alpha}$ levels in synovial fluids taken from untreated patients with chronic inflammatory arthritis are 10^2–10^3 fold lower than those found in carrageenin-induced inflammatory exudates (Higgs & Salmon, 1979). In the latter condition, however, the concentrations of cyclo-oxygenase products in the exudates decrease as the lesion progresses (Higgs & Salmon, 1979). A number of cell types might contribute to the reported spec-

trum of oxygenation of arachidonic acid, i.e. polymorphonuclear (PMN) leucocytes, lymphocytes, platelets, infiltrating macrophages or phagocytic type A synovial lining cells. The latter have been previously suggested to be the major source of $PGF_{2\alpha}$ (Patrono *et al.*, 1976) and PGE_2 (Sturge *et al.*, 1978) local formation in rheumatoid arthritis.

More recently, the ability of partially purified human haematogenous cell populations to metabolize arachidonic acid via the cyclo-oxygenase pathway has been characterized (Morley, Bray, Jones, Nugteren & Van Dorp, 1979). PGE_2 and TXB_2 were found to be the major products formed in all cell types examined, a finding consistent with the results of the present study, showing these compounds to account for more than 60% of the total immunoreactivity measured in synovial fluids from untreated patients.

It has been shown that mouse peritoneal macrophages generate significant amounts of 6-keto-$PGF_{1\alpha}$ (Humes, Bonney, Pelus, Dahlgren, Sadowski, Kuehl & Davies, 1977), while this compound was not found in the culture fluids of guinea-pig and human macrophages (Morley *et al.*, 1979). Our finding that 6-keto-$PGF_{1\alpha}$ is a major component of synovial fluid might suggest a vascular origin of this compound, which represents the major cyclo-oxygenase product in vascular microsomes (Moncada, Gryglewski, Bunting & Vane, 1976). Alternatively, synovial cells might contribute to its formation, in view of 6-keto-$PGF_{1\alpha}$ synthesis by pleural and peritoneal cells (Herman, Claeys, Moncada & Vane, 1979). The absence of any constant ratio between the different oxygenated products of arachidonic acid metabolism detected in 23 synovial fluids obtained from untreated patients is consistent with a heterogeneous cellular origin of these compounds. In addition, the prostaglandin system of the above mentioned cell types known to participate in inflammatory responses can be expected to be variably affected by anti-inflammatory drugs. The results of the present study might be interpreted in accordance with such a prediction. Thus, although synovial concentrations of PGE_2, $PGF_{2\alpha}$, TXB_2 and 6-keto-$PGF_{1\alpha}$ were decreased in all patients following indoprofen treatment, the extent of such a reduction ranged from 36% in the case of 6-keto-$PGF_{1\alpha}$ to 90% in the case of PGE_2. However, obvious ethical reasons limited the possibility of ascertaining the consistency of prostaglandin concentrations over time in the absence of drugs. Moreover, measurement of changes in their actual concentration does not allow for differences in their half lives or differences in factors in the synovial fluid which might affect their presence independently of the rates of synthesis.

A quite distinct pattern of response was observed following steroidal treatment. Thus, only 6-keto-$PGF_{1\alpha}$ concentrations displayed a statistically significant reduction by approximately 35%, i.e. to an extent quite similar to that induced by a nonsteroidal therapy. This finding might suggest the existence of steroid receptors in the cell type(s) involved in PGI_2 synthesis. In discussing the effects of indoprofen and 6-MeP on synovial prostaglandin and TXB_2 concentrations, however, it should be mentioned that while the synovial pharmacokinetics of the former has been described (Caruso *et al.*, 1980), thus supporting a direct in-joint activity, no such information is available on the latter. Moreover, although the short-term study was adequate to observe clinically evident anti-inflammatory effects, it is not known whether similar findings would be obtained under more chronic conditions.

The finding of substantially unaltered PGE_2 and TXB_2 synovial concentrations following 6-MeP treatment raises the question of the mechanism of action of steroids in reducing chronic inflammation. A number of reports in the recent literature suggest that inhibition of prostaglandin synthesis may contribute to the anti-inflammatory properties of steroidal drugs (for review see Gryglewski, 1979). A steroid-induced factor which mimics the anti-phospholipase effect of anti-inflammatory steroids and whose formation requires intact transcription and protein synthesis, has been discovered in the effluent of guinea-pig lungs perfused with dexamethasone (Flower & Blackwell, 1979), in the dialysed supernatant of rat peritoneal leucocytes incubated with hydrocortisone (Carnuccio, Di Rosa & Persico, 1980) and in the particulate fractions of flucinolone-treated rabbit neutrophils (Hirata, Schiffmann, Venkatasubramanian, Salomon & Axelrod, 1980). Although a close relationship was observed between the anti-phospholipase activity and the anti-inflammatory activity of different corticosteroids (Nijkamp, Flower, Moncada & Vane, 1976), no evidence exists that the former action indeed occurs in humans under therapeutic conditions. Failure of 6-MeP to reduce synovial levels of PGE_2 and TXB_2 might reflect at least three distinct mechanisms: (a) the absence of steroid receptors and/or the biosynthetic machinery necessary for protein synthesis in some cell types involved in their production, as in platelets; (b) chronic activation of phospholipase(s) in type A synovial lining cells, actively phagocytosing immune complexes (Kinsella, Baum & Ziff, 1970; Paget & Gibofsky, 1979), might represent a 'steroid-insensitive mechanism' as proposed by Blackwell, Flower, Nijkamp & Vane (1978); (c) in 6-MeP-exposed cells, the reduction of arachidonic acid availability might preferentially divert its further metabolism through the cyclo-oxygenase pathway: such a mechanism would be consistent with unaffected PGE_2 and TXB_2 levels and, possibly, reduced concentrations of lipoxygenase-derived products.

In a few instances (patients 2, 10 and 17), the reduction of synovial PGE_2 levels caused by 6-MeP treatment was associated with comparable reduction of synovial leucocytes. This finding might suggest a primary action of the drug on cell migration as well as a dominant role of PMN leucocytes in prostaglandin production. However, these suggestions are contradicted by opposite changes induced by the same drug in other patients and by lack of a statistically significant correlation between PGE_2 levels and PMN counts before treatment. Consensual changes of the two synovial parameters might perhaps reflect the influence of 6-MeP on chemotactic factors originating via the lipoxygenase pathway of arachidonic acid metabolism.

Finally, the observed decrease in the synovial CH50 levels following 6-MeP treatment is difficult to explain. In experimental animals, it has been shown that the effect of corticosteroids on serum complement levels is clearly biphasic (Atkinson & Frank, 1973). In this study, low doses induced a significant rise in whole complement activity, while higher amounts of cortisone caused a marked fall of many components (Atkinson & Frank, 1973). Surprisingly, in our patients changes in complement levels were observed with corticosteroid doses well below those employed in experimental studies. It remains to be established whether these changes are secondary to the influence of the drug on the disease process or merely reflect a direct effect on the catabolic and/or synthetic rates of complement.

In conclusion, the results of the present study clearly indicate that steroidal and nonsteroidal anti-inflammatory drugs interfere with synovial arachidonic acid metabolism in a quite distinct fashion. Besides contributing to the understanding of the specific anti-inflammatory properties of these drugs, such differences might partially account for the different spectrum of known side-effects, corticosteroids lacking many of the common side-effects currently attributed to ubiquitous inhibition of prostaglandin biosynthesis.

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