

## Change of prostaglandin E level in joint fluids after treatment with flurbiprofen in patients with rheumatoid arthritis and osteoarthritis

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**SUMMARY** The prostaglandin E (PGE) level in the knee joint fluid was determined by radio-immunoassay before and after anti-inflammatory therapy with flurbiprofen in 8 patients with rheumatoid arthritis (RA) and 4 patients with osteoarthritis (OA). The level of PGE in RA joint fluids before the anti-inflammatory treatment was 9.5-1.2 ng/ml and in proportion with the leucocyte count in the joint fluid. A marked decrease of the PGE level was attained with flurbiprofen treatment in 4 patients whose initial PGE levels had been higher than 3.2 ng/ml, while 4 patients with lower PGE levels, namely, 1.4-1.2 ng/ml, did not respond to the drug treatment. In all the OA patients the PGE level was no higher than 1.5 ng/ml and refractory to the anti-inflammatory therapy.

That prostaglandin E (PGE) plays some part in rheumatoid arthritis (RA) has been suggested by several lines of observations. E type prostaglandin appears to stimulate bone resorption in RA joints.<sup>1</sup> The localisation and production of PGE in rheumatoid synovial tissues have been demonstrated by an immunohistochemical method<sup>2</sup> and radio-immunoassay.<sup>3</sup>

PGE has been detected in the joint fluids from RA patients, and the PG level in patients treated with nonsteroidal anti-inflammatory agents is lower than in untreated persons.<sup>3-6</sup> However, these reports do not compare PGE levels in the same patients before and after treatment, except in one case described by Higgs *et al.*<sup>4</sup> Moreover, none of these reports deals with the correlation between drug-induced change of PGE level and extent of inflammatory response in the joint tissues.

The present study was undertaken in an attempt to study the changes of fluid volume, cell contents, and PGE level in the joint fluids from the same patients with RA and osteoarthritis (OA) after treatment

with flurbiprofen, which is a potent inhibitor of the prostaglandin-generating system.<sup>7</sup>

### Materials and methods

#### JOINT FLUIDS

Joint fluid samples from 8 cases with RA and 4 cases with OA of the knee joint were examined (Table 1). Of 8 RA patients 7 had classical or definite RA according to the criteria of the American Rheumatism Association,<sup>8</sup> and 1 had palindromic rheumatism.<sup>9</sup> Firstly, joint fluid was obtained from the knee joint of the patients who had not received steroid or nonsteroidal anti-inflammatory drugs for at least 1 week (I in Table 1). Then, secondly, it was obtained from the same knee joint 1 day after the cessation of anti-inflammatory therapy with flurbiprofen, 240 mg/day for RA and 120 mg/day for OA, for 6 consecutive days (II in Table 1). After the joint fluid had been aspirated, indomethacin was immediately added to it, 10 µg of 0.1% indomethacin solution to 1 ml of the joint fluid, and stored at -20°C until use.

#### CELL COUNTS ON JOINT FLUIDS

Cell counts on the joint fluids from RA patients were

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Table 1 Clinical data and levels of prostaglandin E of RA and OA patients

Case	Age	Sex	Criteria (in RA)	Stage (in RA)	Class	ESR (mm/h)	RA test	CRP	Joint fluid (I)			Joint fluid (II)		
									PGE (ng/ml)	Volume (ml)	Leucocyte (cells/mm <sup>3</sup> )	PGE (ng/ml)	Volume (ml)	Leucocyte (cells/mm <sup>3</sup> )
RA 1	28	F	Classical	III	2	126	+	7+	9.5	20	39 200	2.7	20	46 000
2	66	F	Classical	III	3	151	+	2+	6.1	2	24 000	2.5	1	23 000
3	50	F	Classical	III	3	80	+	2+	5.1	6	18 100	2.9	6	13 100
4	50	M	Palindromic	I	2	85	-	6+	3.2	11	9500	2.0	9	9500
5	47	F	Classical	IV	4	106	+	5+	1.4	15	7000	1.3	15	10 200
6	70	F	Classical	II	2	125	+	2+	1.4	3	4400	1.1	6	18 000
7	65	F	Classical	III	3	30	+	3+	1.2	3	4500	1.2	3	2500
8	39	F	Definite	III	2	52	-	2+	1.2	10	5200	1.2	5	5100
OA 1	52	M	-	-	-	6	-	-	1.5	22	-	1.4	35	-
2	70	F	-	-	-	11	-	-	1.5	10	-	1.3	15	-
3	73	F	-	-	-	15	-	-	1.1	15	-	1.4	30	-
4	47	F	-	-	-	12	-	-	1.0	15	300	1.0	5	300

SI conversion: leucocytes/mm<sup>3</sup> × 10<sup>6</sup> = leucocytes/l.

performed on a haemocytometer after suitable dilution of the fluid with the standard Türk solution.

#### SEPARATION OF PROSTAGLANDINS

The extraction of prostaglandins from the joint fluid and the separation of prostaglandin E were carried out according to the procedure of Jaffe *et al.*<sup>10</sup> with slight modifications.<sup>11</sup>

#### RADIOIMMUNOASSAY OF PROSTAGLANDIN E

The radioimmunoassay was carried out according to the procedure of Ohuchi *et al.*,<sup>11</sup> which was a modification of the methods of Levine *et al.*<sup>12</sup> and Jaffe *et al.*<sup>10</sup> The prostaglandin E fraction obtained by silicic acid minichromatographic separation was completely converted to prostaglandin B by alkaline dehydration, and prostaglandin B<sub>1</sub>-antiserum (Clinical Assays Inc., Cambridge, MA, USA) was used for the quantitative measurement of prostaglandin B.

The cross-reactivity of the prostaglandin B<sub>1</sub>-antiserum to other prostaglandins, as measured by the amounts that provided 50% inhibition of binding, was as follows: prostaglandin B<sub>2</sub> and prostaglandin A<sub>1</sub>, 4 : 1; prostaglandin A<sub>2</sub>, 20 : 1; prostaglandin E<sub>1</sub>, 70 : 1; prostaglandin E<sub>2</sub>, 450 : 1; prostaglandin F<sub>1</sub>α and prostaglandin F<sub>2</sub>α > 10 000 : 1.<sup>11</sup> As this prostaglandin B<sub>1</sub>-antiserum cross-reacted considerably with prostaglandin B<sub>2</sub>, the values measured for prostaglandin E<sub>1</sub> in the presence of prostaglandin E<sub>2</sub> cannot reflect the real value of prostaglandin E<sub>1</sub>. So we expressed the amount of prostaglandin E instead of the amount of prostaglandin E<sub>1</sub>.

In parallel with the sample assay the recovery rate of the added (<sup>3</sup>H) prostaglandin E<sub>1</sub> (5 × 10<sup>-3</sup> μCi, 5,6-<sup>3</sup>H) prostaglandin E<sub>1</sub>, 59 Ci/mmol, the Radiochemical Centre, England) in the joint fluid was

examined and used for the correction of raw data to actual prostaglandin concentration in the joint fluid.

#### OTHER MEASUREMENTS ON RA PATIENTS

The erythrocyte sedimentation rate (ESR), rheumatoid factor (RA test), and C-reactive protein (CRP) were measured as routine laboratory tests for RA patients.

#### Results

PGE levels, fluid volumes, and leucocyte counts in the joint fluids from RA and OA patients are tabulated in Table 1 together with the ESR, results of the RA test, and serum CRP values. The PGE levels before the drug treatment of RA patients varied over a comparatively wide range from 9.5 to

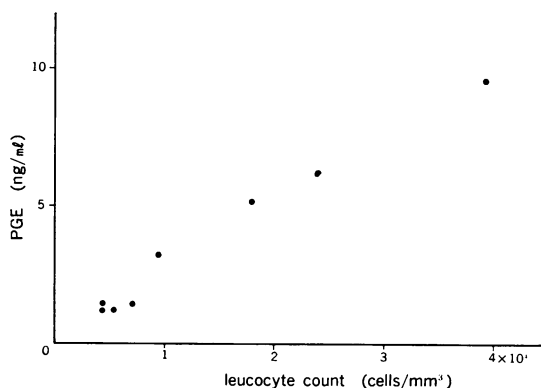


Fig. 1 Relation between prostaglandin E (PGE) and leucocyte count in the joint fluids of RA patients before and after the anti-inflammatory therapy with flurbiprofen. The levels of PGE (ng/ml) were plotted against leucocyte count (cells/mm<sup>3</sup>).

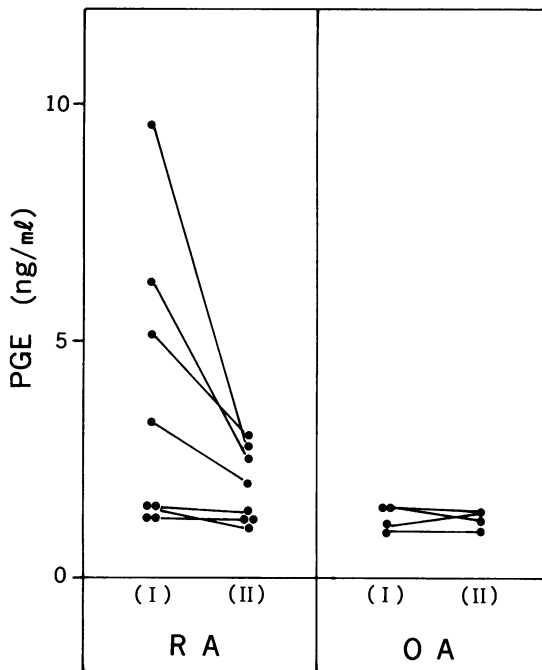


Fig. 2 Changes in the levels of prostaglandin E (PGE) in the joint fluids of RA and OA patients before and after the anti-inflammatory therapy with flurbiprofen. (I) and (II) = joint fluid (I) and (II) obtained before and after the anti-inflammatory therapy, respectively.

1.2 ng/ml, while the levels of OA patients fell into a comparatively low and narrow range from 1.5 to 1.0 ng/ml.

During the anti-inflammatory therapy with flurbiprofen the PGE levels in RA patients with the initial PGE values higher than 3.2 ng/ml declined by 72–38%, while the remaining 4 cases with the initial PGE levels not higher than 1.4 ng/ml kept their initial levels in spite of the drug treatment (Fig. 1).

The PGE in OA patients, whose PGE levels in the joint fluids were also at a low level, namely 1.5–1.0 ng/ml, did not respond to the therapy at all.

No significant change was observed in respect of the cell counts or volume of the joint fluid after the therapy with flurbiprofen either in RA or in OA.

## Discussion

Inflammation of articular tissue in OA appears to be due mainly to mechanical injury of the tissues caused by deformation of the joint structures, namely, bone, cartilage, and ligaments. On the other hand immune

complexes are thought to play an important role in the pathogenesis of rheumatoid synovitis.

Effusion of joint fluids in these joint diseases is thought to be derived from inflammatory lesions of articular tissues. However, the exact mechanisms mediating vascular permeability in these diseases are not well understood yet. In a variety of clinical and experimental inflammations E type prostaglandin generated in injured tissues is considered to be of importance in the mediation of hyperalgesia, vasodilatation, increased vascular permeability, and fever.<sup>13</sup> In the present study, however, no apparent correlation was observed in RA between PGE levels in the joint fluid and various parameters generally accepted as indicators of severity of RA inflammation such as the ESR, serum CRP, and volume of the joint fluids aspirated from affected knee joints, apart from cell counts (Table 1, Fig. 1).

Data on PGE levels and leucocyte counts in joint fluids of rheumatic diseases presented by Robinson and Levine<sup>14</sup> also include findings suggesting that there is no correlation between these 2 parameters. The inflammatory group designated by them on the basis of cell counts 1000/mm<sup>3</sup> or more in the joint fluids showed extensive variation in PGE levels in the joint fluids, with very low values that could not be distinguished from those of the noninflammatory (<1000 cells/mm<sup>3</sup>) group.

In addition, our data on the influence of flurbiprofen to the joint fluids also appear to be inconsistent with the general concept of the role of PGE in inflammation. When the drug was administered to RA patients with a high level of PGE in joint fluids (patients 1, 2, 3, and 4), the PGE levels decreased markedly without affecting either effusion of joint fluids or emigration of cells into the fluids (Table 1). Moreover in half (4) the RA cases and all the 4 OA cases effusion of joint fluids took place in spite of a comparatively low level in PGE in the fluid, but in addition the PGE level and effusion of joint fluid were refractory to anti-inflammatory therapy with flurbiprofen.

Trang *et al.*<sup>6</sup> report, though without including precise data, that all patients treated with various non-steroidal anti-inflammatory drugs still developed acute exudative arthritis in spite of treatment which led to much lower levels of PG's in joint fluid than in untreated patients. The present study, based on the observations made on same patients before and after anti-inflammatory treatment, has provided new evidence in favour of such concepts.

Recent novel studies by Samuelsson<sup>15</sup> have shown that the leucotriens are produced from arachnoidic acid by leucocytes through the lipoxigenase pathway. Leucotrien B has an extremely potent chemotactic

activity.<sup>16</sup> The lipoxygenase pathway is not affected by aspirin-like drugs such as flurbiprofen. The ineffectiveness of our therapy with flurbiprofen on cell counts may therefore be explained along these lines.

Ferreira<sup>13</sup> reported that prostaglandins do not cause overt pain but hyperalgesia; thus, they can sensitise pain receptors. Our clinical impression is that the arthralgia of RA is suppressed in varying degrees after anti-inflammatory therapy with non-steroidal drugs. The significance of PG synthesis inhibitors in anti-inflammatory therapy might be evaluated on the basis of their analgesic effect.

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