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Plasma levels and urinary excretion of prostaglandins in patients with rheumatoid arthritis

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SUMMARY No significant differences were found in plasma concentrations and urinary excretion of prostaglandin E_2 (PGE_2), 6-keto-prostaglandin-$F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and thromboxane B_2 (TxB_2), between rheumatoid arthritis patients and controls. However, urinary excretion of PGE_e and 6-keto-$PGF_{1\alpha}$ tended to be greater and plasma levels of TxB_2 lower in rheumatoid arthritis. Plasma concentrations and urinary excretion showed no marked circadian variation, although night or morning values were slightly lower. Plasma and urine prostaglandins do not correlate with clinical symptomatology in rheumatoid arthritis.

Key words: Prostaglandins, Rheumatoid Arthritis, Collagen Diseases, Inflammation.

INTRODUCTION

The central role of prostaglandins (PGs), metabolites of arachidonic acid, in inflammation is widely accepted (1,2). The action of prostaglandins was originally believed to be entirely pro-inflammatory. There are reports, however, on their anti-inflammatory properties, especially in chronic inflammatory models in experimental animals. Especially prostaglandins of the E-group have been shown to be anti-inflammatory. It is highly probable that this action is mediated through cyclic nucleotides. The anti-inflammatory effects of prostaglandins include: inhibition of lymphocyte mitogenesis, cell-mediated cytotoxicity and lymphokine release, inhibition of the spreading, adherence and migration of the macrophages as well as their differentiation, lysosomal enzyme secretion, and release of mediators on inflammation from mast cells (see 3). These findings have led to postulations that endogenous prostaglandins may exert an important, probably regulatory, feedback function in chronic inflammatory processes also in man.

Though studies on the relationships

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between prostaglandins and inflammation have increased, the possible role of prostaglandins in rheumatoid arthritis has so far been the object of few investigations (4,5,6). Prostaglandins have generally been regarded as local modulators, and in rheumatoid arthritis elevated levels have been measured in synovial fluid. However, rheumatic inflammation is a systemic disease which warrants the measurement of prostaglandins in other biological fluids as well, especially because there have been no reports on these assays under standardized conditions. The present study is one in a series of investigations in which we have studied the relationships of some prostaglandins with rheumatoid arthritis and its clinical symptoms.

PATIENTS AND METHODS

The study covered 14 hospital patients, of whom nine were women and five men. The ages of the patients with rheumatoid arthritis (n = 7) ranged from 20 to 61 years (mean 38), and of the control subjects (n = 7) from 25 to 58 (mean 46). The patients with rheumatoid arthritis fulfilled the ARA criteria of probable or definite rheumatoid arthritis. They had active disease with synovitis in several joints and laboratory signs of inflammation. Their mean ESR was 55 (40-77) and haemoglobin 119 (96-140 g/l). The patients serving as control subjects suffered from soft-tissue rheumatism and/or arthritic complaints. They had no clinical or laboratory signs of any inflammatory disease. They were admitted to the hospital for physical treatment or, initially, for diagnostic purposes.

Prior to the study, the patients abstained for at least a week from all nonsteroidal anti-inflammatory drugs. The only analgesic permitted during the period preceding the study was paracetamol. During the study the patients took normal meals and their physical activity was not limited. Blood samples were taken at 3 h-intervals into cooled test tubes containing EDTA-ASA solution (10 mg disodium EDTA and 0.18 mg ASA per 10 ml of blood). The sample was mixed gently and was then centrifuged immediately at 500 g for 15 minutes at a temperature of +4°C. The plasma was kept at −20°C until

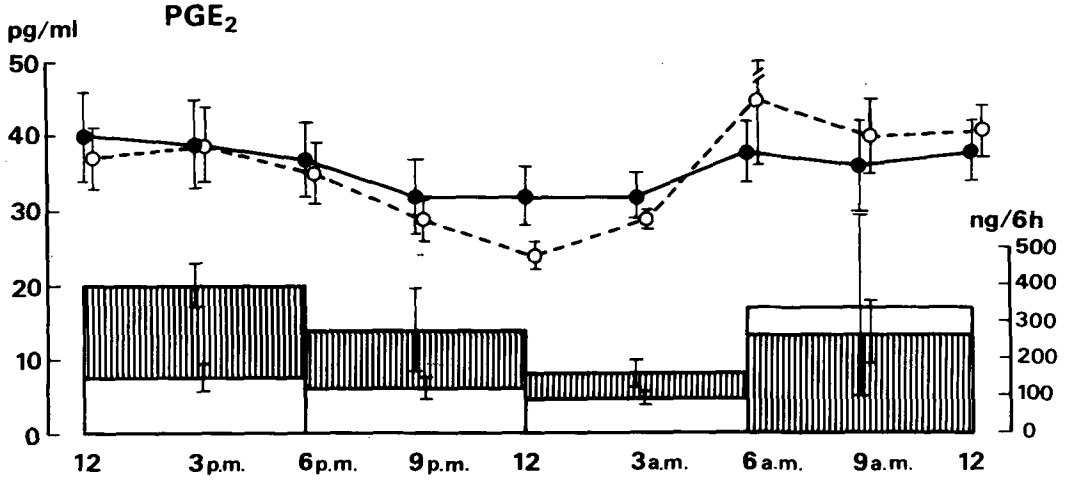

Fig. 1: Concentrations of PGE_2 in plasma measured at 3-h intervals and excretion of PGE_2 into urine during 6-h periods in rheumatoid arthritis patients (closed circles and shadowed columns) and in control subjects (open circles and open columns). Mean ± S.E., n = 7, for plasma, and 4 (control subjects) and 5 (patients), for urine.

assayed; the stability of various PGs has been demonstrated at this temperature (7).

Urine was collected over a 24 h-period in 6 h-collection sequences and was frozen immediately at −20°C. Samples were purified using XAD-2 resin. PGs were eluted using a mixture of isopropanol-ethyl acetate (1 :5) and finally with ethyl acetate. Plasma and urine PGE_2, 6-keto-$PGF_{1\alpha}$, the stable metabolite of prostacyclin (PGI_2), and thromboxane (TxB_2), the stable metabolite of thromboxane A_2, were measured by radioimmunoassay, using a double antibody technique and antisera from the Pasteur Institute (Paris, France). The cross-reactivity of the antisera against the most common arachidonic acid metabolites has been tested by the manufacturer and shown to be negligible. The assay methods were mainly those of Jaffe (8), with slight modifications described here and in more detail later (9). Student's t-test was used for the statistical analysis.

RESULTS

There were no significant differences between the two sexes in the plasma concentrations of PGE_2, 6-keto-$PGF_{1\alpha}$ or TxB_2 either in the rheumatic or in the non-rheumatic group. Therefore, the values of women and men were combined.

In some cases the urinary excretion of PGE_2 in men showed very high values, probably due to seminal fluid contamination. Therefore, only the female values are presented. On the other hand, the excretions of 6-keto-$PGF_{1\alpha}$ and TxB_2 were similar in women and men, and their values were combined.

The plasma concentrations and urinary excretion of PGE_2 are given in Fig. 1. No clear-cut hourly variation was found in the plasma concentrations, although the values during the night (between 9 p.m. and 3 a.m.) were slightly lower in both the rheumatoid arthritis patients and the controls. There

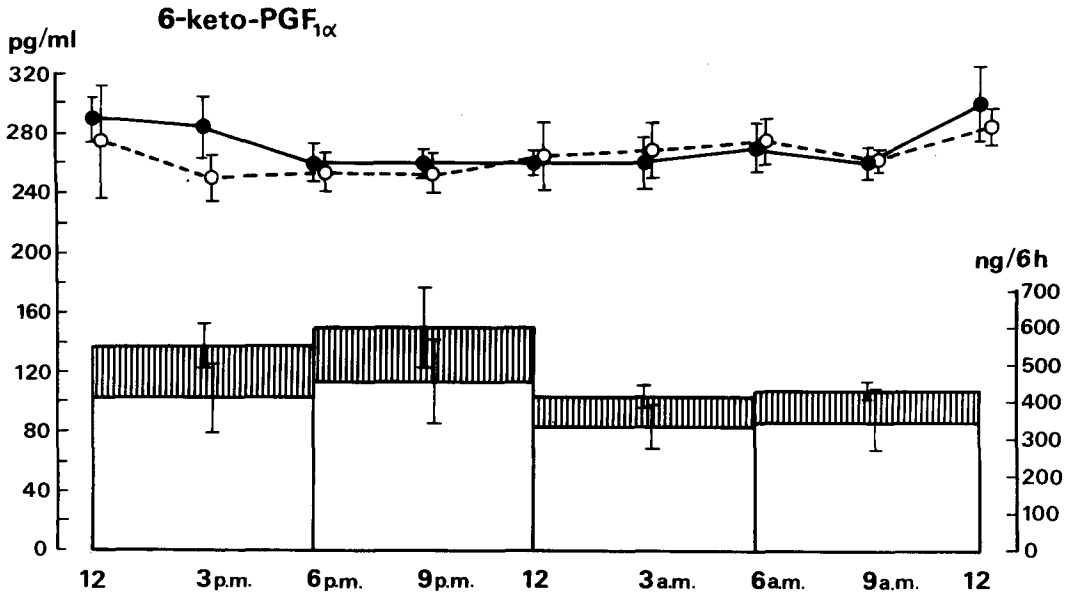

Fig. 2: Concentrations of 6-keto-$PGF_{1\alpha}$ in plasma and excretion of 6-keto-$PGF_{1\alpha}$ into urine in rheumatoid arthritis patients (closed circles and shadowed columns) and in control subjects (open circles and open columns). Means ± S.E., n = 7. Intervals and urine collection periods as in Figure 1.

were no significant differences between these two groups. The mean values of all measurements were 36.1 ± 1.5 (mean $\pm$ SE) pg/ml for the rheumatoid arthritis patients and 35.5 ± 1.7 pg/ml for the control subjects. Urinary excretion of PGE_2 was lowest between midnight and 6 a.m., but the difference was significant only in the group of rheumatoid arthritis patients. During three of the four collection periods, rheumatoid arthritis patients showed higher excretion, but significantly only from noon to 6 p.m. ($p < 0.01$).

The plasma concentrations and urinary excretion of 6-keto-$PGF_{1\alpha}$, the stable metabolite of prostacyclin, are shown in Fig. 2. There was no hourly variation in either group, nor did the rheumatoid arthritis patients differ from the control subjects. The mean values of all measurements were 274 ± 5 pg/ml for the rheumatoid arthritis group and 267 ± 6 pg/ml for the control group.

In both groups, the smallest excretion of 6-keto-$PGF_{1\alpha}$ into urine occurred during the morning periods (from midnight to noon). The urinary excretion of 6-keto-$PGF_{1\alpha}$ was slightly, but not significantly, greater in the patients with rheumatoid arthritis during all four collection periods.

The plasma concentrations and urinary excretion of TxB_2, the stable metabolite of TxA_2, are given in Fig. 3. No clear-cut hourly variation was observed in the plasma concentrations, although the lowest values in the rheumatoid arthritis patients tended to be in the morning hours. But each time the mean plasma TxB_2 values of the control group were considerably higher than those of the rheumatoid arthritis group. Owing, however, to the large variation of the individual values within each group, the differences between the groups were not statistically significant, and the mean values of all measurements were 84 ± 8 pg/ml for the patients with rheumatoid arthritis and

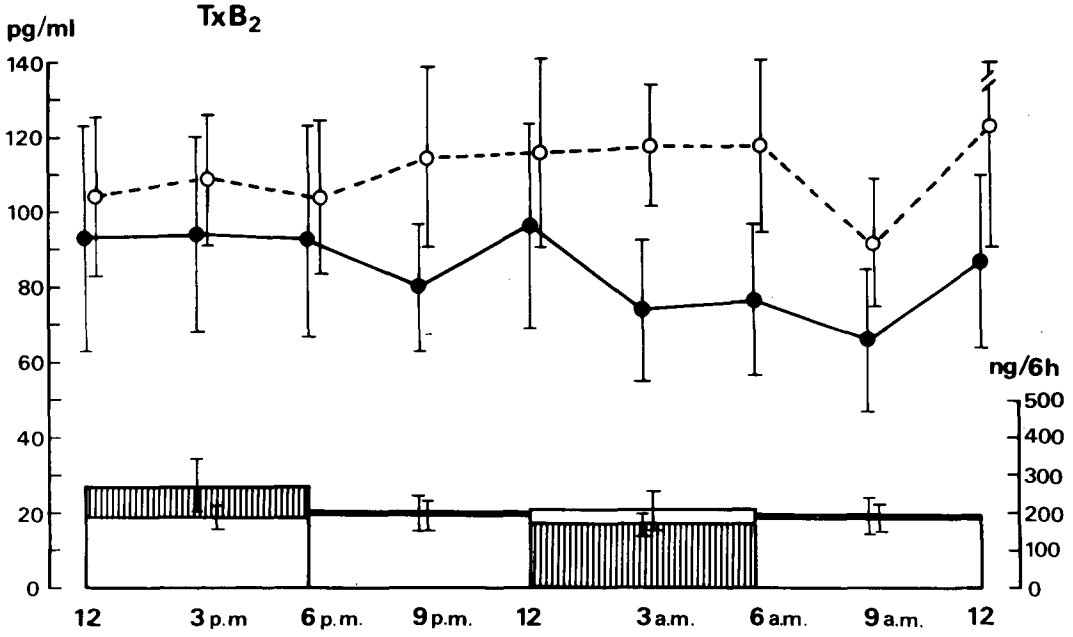

Fig. 3: Concentrations of TxB_2 in plasma and excretion of TxB_2 into urine in rheumatoid arthritis patients (closed circles and shadowed columns) and in control subjects (open circles and open columns). Means $\pm$ S.E., n = 7. Intervals and urine collection periods as in Figure 1.

111 ± 7 pg/ml for the control subjects. In both groups, the urinary excretion of TxB_2 was similar during every collection period.

The total 24 h-excretion of the three prostaglandins into urine (Table 1) tended to be greater in the women with rheumatoid arthritis than in the control women. The differences were, however, statistically insignificant. No correlation was found between the plasma levels or urinary excretion of prostaglandins and the clinical or laboratory (ESR, haemoglobin, Latex, Waaler-Rose) activity of the disease.

DISCUSSION

In the present study, the plasma concentrations and urinary excretions of three important arachidonic acid metabolites, PGE_2, 6-keto-$PGF_{1\alpha}$ and TxB_2, were observed in hospitalized rheumatoid arthritis patients and control subjects for 24 hours under standardized conditions. Even under these well controlled conditions, there were marked individual variations in the parameters measured. This might be the reason why the differences did not reach statistical significance, even though tendencies to higher urinary excretion of PGE_2 and 6-keto-$PGF_{1\alpha}$, and lower plasma TxB_2 values were found in the rheumatoid arthritis patients. Another reason may have been the small number of patients in each group.

Even though the concentrations of prostaglandins in the synovial fluids of rheumatoid arthritis patients are highly increased (10,11), this phenomenon does not seem to be reflected an increase in the plasma concentrations or a considerable increase in the excretion into urine.

Another unexpected finding was the lack of a clear-cut circadian rhythm both in the plasma prostaglandin concentrations and in their excretion into urine. However, a tendency towards lower values of PGE_2 and TxB_2 plasma concentrations and PGE_2 and 6-keto-$PGF_{1\alpha}$ excretion during the night or morning hours was again observed. The lack of a significant circadian rhythm in plasma and urine prostaglandin levels in rheumatoid arthritis patients is contradictory to the suggested important role of prostaglandins in the symptomatology of rheumatic inflammation, which shows a marked circadian variation.

On the basis of our results it seems evident that the levels of arachidonic acid metabolites in plasma or in urine cannot be used as indicators of the activity of rheumatic inflammation, though there may be a manifold increase of their local formation at the site of inflammation. This agrees with the

Table I *Daily urinary excretion (ng/24 h) of PGE_2, 6-keto-$PGF_{1\alpha}$ and TxB_2 in rheumatic and non-rheumatic patients. Means ± S.E. are given.*

		PGE_2	6-keto-$PGF_{1\alpha}$	TxB_2
Patients with rheumatoid arthritis				
females	(5)	1.1 ± 0.2	2.0 ± 0.2	0.8 ± 0.5
males	(2)	13.7 ± 11.2	1.9 ± 0.1	0.8 ± 0.2
both sexes	(7)	—	2.0 ± 0.1	0.8 ± 0.2
Control subjects				
females	(4)	0.7 ± 0.3	1.2 ± 0.3	0.8 ± 0.2
males	(3)	37.7 ± 21.4	2.0 ± 0.6	0.8 ± 0.3
both sexes	(7)	—	1.5 ± 0.3	0.8 ± 0.2

suggestion of Granström (12) that the correlation between plasma prostanoids and their formation in different organs is low. The excretion of these substances into urine seems to be somewhat more compatible with the clinical situation than are the concentrations in plasma. These results do not exclude the possibility that other arachidonic acid metabolites may better reflect the clinical situation and the activity of the disease.

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