

SCIENTIFIC PAPERS

Hypoxanthine, xanthine, and urate in synovial fluid from patients with inflammatory arthritides

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Abstract

As nucleotide catabolism increases during tissue injury the appearance of purine metabolites in inflamed synovial fluid might be of value in understanding the joint damage in inflammatory arthritides. In this study, therefore, synovial and plasma concentrations of hypoxanthine, xanthine, and urate in 16 patients with rheumatoid arthritis (three with psoriatic arthropathy) were analysed. It was found that their plasma concentrations of hypoxanthine were greater than those of a reference group of healthy subjects. The synovial fluid concentrations of hypoxanthine, xanthine, and urate were higher than corresponding concentrations in plasma. Positive correlations were found between the respective plasma and synovial fluid values of xanthine and urate. These findings indicate a local enhanced purine metabolism in inflamed joint tissue and diffusion of oxypurines from joint cavity to plasma. No relation was found between measured metabolites and disease duration, radiological joint findings, or synovial fluid cells. Except for a weak correlation between plasma urate and serum haptoglobin, measured purine metabolites were not related to laboratory measures of systemic inflammation.

Joint damage is an important consequence of rheumatoid arthritis and other inflammatory arthritides and accounts for functional impairment and disability. The traditional view is that inflammation is the major mechanism behind the joint destruction. Therefore, the main attraction has been directed towards various inflammatory mediators. In clinical practice several indices of inflammatory activity, such as erythrocyte sedimentation rate and acute phase plasma proteins, have been considered as valuable markers. These estimates of disease activity cannot be considered as reliable predictors of progressive joint damage, however.¹⁻³ Thus novel markers for an understanding of the disease process are urgently needed. It has been suggested that joint damage may result from a series of pathological changes unrelated to the inflammation.⁴⁻⁸ Metabolically unfavourable effects on the joint structures might be secondary to altered nutrition or ischaemia, for example, and could be reflected by purine metabolites. In a previous study increased synovial fluid concentrations of hypoxanthine were reported in patients with rheumatoid arthritis.⁹ In an attempt to elucidate further the nucleotide metabolism within the inflamed joint cavity we

measured the synovial fluid concentrations of the oxypurines hypoxanthine, xanthine, and urate in patients with inflammatory arthritides. Data obtained were related to the inflammatory activity and clinical symptoms, disease duration, and radiological joint changes.

Patients and methods

Ten women and six men with destructive and symmetric polyarthritis were included in the study. Eleven patients fulfilled the criteria of the American Rheumatism Association¹⁰ for classical rheumatoid arthritis. Two patients had juvenile rheumatoid arthritis. Three patients had seronegative polyarthritis associated with psoriasis. Their mean age was 42.6 years (range 21-68) and the mean disease duration was nine years (range six months to 18 years). All patients were taking non-steroidal anti-inflammatory drugs or aspirin and seven patients were treated with sulphasalazine. Table 1 presents the clinical data. Subjective clinical symptoms—morning stiffness and joint pain in the aspirated knee—and objective symptoms of active arthritis—degree of heat in the skin and grade of knee effusion—were estimated. These symptoms were graduated as mild, slight, moderate, or severe. Seventeen healthy laboratory staff (11 women, six men) who were not taking any drugs served as controls. Their mean age was 42 years (range 24-64).

Synovial fluid specimens were collected from inflamed knee joints by needle aspiration into EDTA tubes, when therapeutically indicated. In one patient knee joint aspirations were done twice (case Nos 1, 16) and in another patient both knees were aspirated (Nos 9, 10). The synovial fluid samples were immediately centrifuged (20 minutes, 2000 g), and 2 ml of the supernatants was treated with 0.3 ml of perchloric acid (4 mol/l) to remove protein. The excess perchloric acid in the supernatant was neutralised with a mixture of crystalline potassium carbonate and potassium phosphate. The specimens were centrifuged for five minutes and the supernatant kept frozen at -20°C until analysed. EDTA plasma was treated in the same way.

Urate was analysed by reversed phase high performance liquid chromatography by direct injection of the neutralised supernatant. Hypoxanthine and xanthine in the supernatant were analysed similarly after precleaning on small cation exchange columns. These columns, 0.35×2 cm, were packed with Bio-Rad AG 50W×12, 200-400 mesh in H⁺ form. After application and rinsing the purines were eluted with 1.0 ml of phosphoric acid, 4 mol/l.

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Table 1 Clinical data, roentgenological findings, and synovial fluid cells in patients with inflammatory arthritides

Case No	Age (years)	Sex	Disease duration (years)	x Ray‡	Diagnosis	Synovial fluid cells		
						Leucocytes ($10^{12}/l$)	PMN§ (%)	MN§ (%)
1*	47	F	10	No	PsA§	22.6	78	22
2	52	M	4	No	RA§	12.6	86	14
3	29	F	10	No	RA	10.1	6	94
4	21	F	18	No	JRA§	16.2	6	94
5	25	F	3	ND	RA	33.8	75	25
6	32	F	9	No	RA	15.8	65	35
7	44	M	9	Yes	PsA	13.1	67	33
8	43	M	10	Yes	PsA	5.0	32	68
9†	30	F	4	No	RA	11.7	47	53
10†	30	F	4	No	RA	12.6	47	53
11	67	F	14	Yes	RA	4.5	4	96
12	41	M	9	Yes	RA	13.0	67	33
13	52	M	9	ND	RA	4.6	55	45
14	32	M	16	No	RA	8.1	77	23
15	31	F	18	No	JRA	5.2	48	52
16*	47	F	10	No	PsA	4.5	57	43
17	67	F	1	No	RA	21.5	38	62
18	68	F	0.5	No	RA	19.2	77	23

*Joint aspiration from the same knee of the same patient with a four month interval.

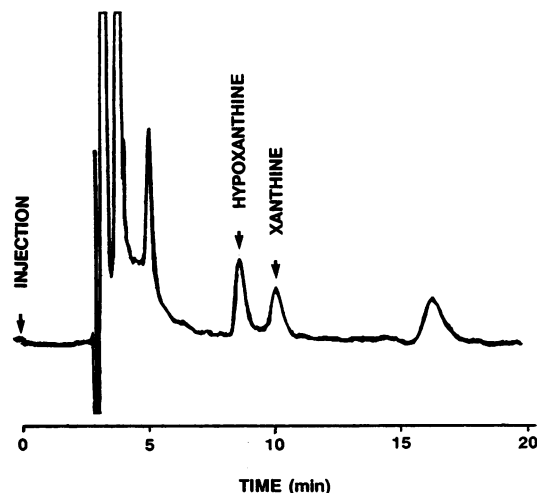
†Left (No 9) and right (No 10) knee joint aspiration from the same patient.

‡x Ray=radiographic destruction in the knee joint.

§PsA=psoriatic arthritis; RA=rheumatoid arthritis; JRA=juvenile rheumatoid arthritis; PMN=polymorphonuclear; MN=mononuclear.

The chromatographic system used consisted of a pump (Constametric model III, LDC, Florida), an ultraviolet absorbance detector (Spectromonitor III, LDC, Florida), a column (300×3.9 mm) packed with μ Bondapak C18 (Waters Associates, Milford, MA 01757, USA), and a loop injector, 20 μ l. Potassium phosphate buffer, 0.2 mol/l, pH 5.9, was used as the mobile phase and the flow was 1 ml/min. Hypoxanthine, xanthine, and urate in the chromatogram (fig 1) were identified by comparing the peaks with the retention times for the pure substances and by the peak shift technique after adding the enzyme xanthine oxidase (Boehringer-Mannheim, Mannheim, Germany) to the specimens. Measurement of the oxypurines in synovial fluid and plasma was performed from standard curves of hypoxanthine, xanthine, and urate produced by peak height measurements of standard samples with known concentrations. The hypoxanthine and xanthine concentrations in healthy controls were 1.1 (SD 0.5) μ mol/l and 0.4 (0.1) μ mol/l, respectively. Further details of the chromatographic technique for oxypurine measurements are presented elsewhere.¹¹

Figure 1 A typical high performance liquid chromatogram of hypoxanthine and xanthine from synovial fluid which has been deproteinised and precleaned on a cation exchange column. For chromatography conditions see text.



The inflammatory activity was estimated by measuring erythrocyte sedimentation rate according to Westergren (normal <15 mm/h), C reactive protein (normal <10 mg/l), and haptoglobin (normal 0.2–1.4 g/l). Cell numbers and differential cell counting were performed on synovial fluids with routine methods.

STATISTICS

For statistical evaluation of differences between groups Student's *t* test was used. Linear correlation analysis of data was used as indicated.

Results

Table 2 presents the plasma and synovial fluid concentrations of hypoxanthine, xanthine, and urate in patients with inflammatory arthritides. The mean synovial fluid concentrations of all oxypurine metabolites were higher in synovial fluid than in plasma. The plasma concentrations of hypoxanthine, xanthine, and urate were slightly higher in the patient group than in the healthy controls (table 2), but only the increase in plasma hypoxanthine reached significance ($p < 0.001$). The measured metabolites in plasma or synovial fluid were not related to sex, age, or disease duration.

A weak relation was noted between the estimated effusion of knee joint and synovial fluid concentrations of hypoxanthine ($r = 0.56$, $p < 0.05$) and xanthine ($r = 0.052$, $p < 0.05$) respectively. Other subjective or objective clinical estimates of the arthritic condition were not related to the oxypurine metabolites in synovial fluid or plasma, except for a weak relation between morning stiffness and plasma urate ($r = 0.58$, $p < 0.05$). The x ray findings were discrete in most patients and only four had cartilage reduction but no destruction of the knee joint. The measured oxypurine concentrations were not related to the x ray findings.

The median erythrocyte sedimentation rate was 27 mm/h (range 5–120) and median C reactive protein 19 mg/l (range <10–50). Mean (SD) haptoglobin was 3.2 (1.0) g/l (range 1.28–4.24). Except for a weak correlation between synovial fluid urate and serum haptoglobin ($r = 0.52$, $p < 0.05$) we found no relation between the concentrations of oxypurine metabolites and laboratory signs of systemic inflammatory activity. The local joint inflam-

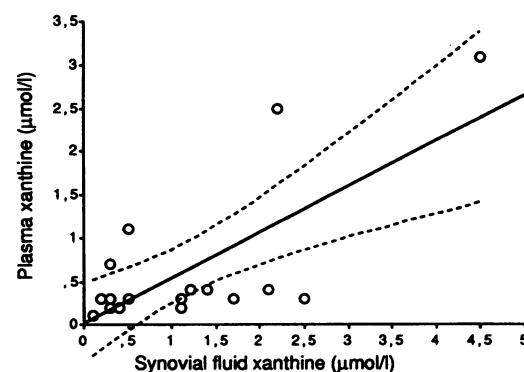


Figure 2 Concentration of xanthine in synovial fluid versus the plasma concentration of xanthine in patients with inflammatory arthritides ($r = 0.71$, $p < 0.001$).

Table 2 Mean (SD) hypoxanthine, xanthine, and urate concentrations in plasma and synovial fluid samples from 16 patients with inflammatory arthritides and in plasma from 17 control subjects

Metabolite	Controls (n=17)	Patients (n=16)		Correlation coefficient [†]
	Plasma	Plasma (μmol/l) (n=17)	Synovial fluid (μmol/l) (n=18)	
Hypoxanthine	1.1 (0.5)*	2.1 (1.5)*	2.3 (1.8)	0.11
Xanthine	0.4 (0.1)	0.7 (0.9)	1.2 (1.1)	0.71*
Urate	194 (41)	215 (64)	229 (74)	0.76*

* $p < 0.001$.

†Correlation coefficients between plasma and synovial fluid concentrations of the respective metabolite.

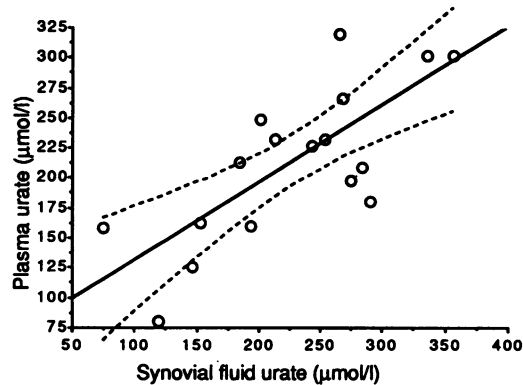


Figure 3 Concentration of urate in synovial fluid versus the plasma concentration of urate in patients with inflammatory arthritides ($r=0.76$, $p < 0.001$).

matory activity was estimated by the presence of white blood cells and differential counting (table 1). The numbers of polymorphonuclear or mononuclear cells were not related to the synovial fluid concentrations of oxypurine metabolites.

We found significant correlations ($p < 0.001$) between the plasma and synovial concentrations of xanthine (fig 2) and urate (fig 3) respectively. The hypoxanthine concentrations in synovial fluid were not related to those in plasma (table 2).

Discussion

By using high performance liquid chromatography we found increased plasma concentrations of the oxypurine hypoxanthine in patients with rheumatoid arthritis and other inflammatory arthritides; this is in accordance with a previous report.⁹ We also found that the synovial fluid concentrations of hypoxanthine, xanthine, and urate were greater than the corresponding plasma concentrations. An increase of hypoxanthine in inflamed synovial fluid in relation to plasma has previously been noted in patients with rheumatoid arthritis but not in osteoarthritis.⁹ These data suggest that the inflamed joint is a compartment with an enhanced nucleotide metabolism and that diffusion of purine metabolites occurs from the joint to the blood circulation. The strong correlations found between xanthine and urate concentrations in plasma and synovial fluid, respectively, suggest that the inflamed joint contributes significantly to the circulating concentrations of measured oxypurines. The concentrations of hypoxanthine, xanthine, or urate in plasma or synovial fluid were not related to the disease duration or

x ray findings of the joints. Except for a weak correlation between synovial fluid urate and serum haptoglobin we found no relation between the laboratory markers of inflammatory activity and oxypurine concentrations in plasma or synovial fluid.

During catabolism hypoxanthine is converted to xanthine and then to urate by xanthine oxidase. If this enzyme was absent in the joint, xanthine and hypoxanthine would be the end products of purine metabolism of the joint, rather than urate. Xanthine oxidase was previously thought to be almost exclusively found in the liver and intestinal mucosa in man.¹² The presence of increased concentrations of xanthine oxidase was recently reported in the synovial tissue from patients with rheumatoid arthritis.¹³ In our study the ratio between synovial fluid and plasma concentrations of xanthine and urate was found to be >1.0 , which may support the presence of xanthine oxidase within the inflamed joint cavity. The increases of the synovial fluid concentrations were fairly small, however, compared with the plasma concentrations and the differences may partly be due to analytical differences in processing two different biological materials. One possible source of xanthine oxidase in the inflamed joint cavity might be the large number of invading inflammatory cells. We found no correlation between the cell counts or the appearance of oxypurines in synovial fluid, however.

Purine metabolites in inflamed synovial fluid may result from adenosine 5-triphosphate catabolism in senescent joint cells like neutrophils, lymphocytes, or cells from pannus. Degradation of DNA and other cellular substances containing purine metabolites may also occur in necrotic lesions of the inflamed synovia. Finally, the appearance of increased concentrations of purine bases in synovial fluid may be due to ischaemia in joint structures.

It has been known for several years that increased catabolism of nucleotides occurs during tissue hypoxia.^{14 15} Hypoxanthine in plasma has been proposed to be a good indicator of tissue hypoxia.^{16 17} Local accumulation of degradation products from the nucleotide pool is also reported to occur in ischaemic organs. Clinical studies in patients with cerebral ischaemia have reported considerable increases of hypoxanthine and xanthine concentrations in the cerebrospinal fluid.¹⁸ During persistent synovial inflammation with effusion, hypoxia in the synovial cavity is reported.¹⁹ Reduction of the oxygen tension has been attributed to pressure changes induced by exercise. The blood supply to the synovium is supposed to be transiently reduced owing to the increased pressure. Thus the appearance of increased synovial fluid concentrations of hypoxanthine, xanthine, and urate may reflect ischaemic damage in the joint due to increased joint pressure. In support of this hypothesis we found that the estimated effusion of the investigated knee joint correlated with its synovial fluid concentrations of hypoxanthine and xanthine. The tissue damage associated with temporary ischaemia may partly be dependent on the production of free radicals by the

xanthine/xanthine oxidase system.²⁰⁻²² In this context it is worth noting that thiol groups found, for example, in penicillamine and gold compounds interfere with this free radical generating system.

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