Circulating inhibitor bound elastase in patients with ankylosing spondylitis and rheumatoid arthritis and the influence of sulphasalazine treatment

NILS FELTELIUS AND ROGER HÄLLGREN

From the Department of Internal Medicine, University Hospital, Uppsala, Sweden

SUMMARY The plasma concentration of granulocyte elastase in complex with α_1 proteinase inhibitor was determined in 42 patients with ankylosing spondylitis (AS) and 33 patients with rheumatoid arthritis (RA). Significantly raised levels of plasma elastase were found in patients with RA, whereas patients with AS had normal values. No correlation was seen between the elastase values and erythrocyte sedimentation rate (ESR), serum haptoglobin, immunoglobulins or polymorphonuclear cell (PMN) count in either of the patient groups. A correlation was found between the Ritchie index and plasma elastase in patients with RA. After three months' treatment with sulphasalazine a clinical improvement was seen and this paralleled a reduction of the acute phase reaction in both patient groups. A reduction of the circulating elastase values was seen in the patients with RA, whereas no change was seen in patients with AS.

Key words: acute phase reaction, Ritchie index.

Elastase is one of the neutral proteases contained within the primary granules of the polymorphonuclear granulocytes.¹ PMN elastase possesses elastolytic and collagenolytic properties and is thought to contribute to tissue damage in several inflammatory diseases.² In addition to its effects on connective tissue components, PMN elastase also attacks plasma proteins like immunoglobulins, clotting factors, and complement components, properties that may further contribute to its harmful effects in rheumatic diseases.² The activity of the proteinases is controlled by potent proteinase inhibitors. Elastase is inhibited by α_1 proteinase and to a lesser extent α_2 macroglobulin.¹ In this and previous studies plasma elastase has been used as a marker for PMN activation.³ Other cells, including platelets and macrophages, contain enzymes with elastolytic activity, but these enzymes appear to be immunologically unrelated to PMN elastase.⁴ Measurement of plasma elastase in complex with α_1 proteinase inhibitor is considered to give a good reflection of the circulating plasma levels of PMN elastase.⁵

Patients with AS and other HLA-B27 associated arthritides have been reported to have neutrophils with enhanced reactivity.⁶⁻⁷ We have previously reported increased circulating levels of a specific eosinophil cationic granule protein (ECP) in patients with RA and AS as a possible sign of eosinophil activation.⁸⁻¹⁰ Raised circulating levels of granule proteins from neutrophils and eosinophils could either reflect an increased turnover of cells with subsequent release of proteins or an enhanced readiness to secrete proteins as a sign of activation. In this study we wanted to elucidate the neutrophil involvement in patients with RA and AS by measuring plasma elastase. As we previously had noticed a reduction of the ECP levels during treatment with sulphasalazine another aim of this study was to elucidate whether circulating elastase levels were influenced in a similar way.

Patients and methods

PATIENTS

Forty two patients (six women, 36 men) with ankylosing spondylitis according to the New York criteria¹¹ and 33 patients (18 women, 15 men) with rheumatoid arthritis according to the American Rheumatism Association criteria¹² were included in

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Correspondence to Dr Nils Feltelius, Section of Rheumatology, Department of Internal Medicine, University Hospital, S-751 85 Uppsala, Sweden.

the study. The mean age (range) in the AS group was 40 (19–57) years and in the RA group 53 (16–75) years. The mean duration of disease (range) in the AS group was 11 (1–30) years and in the RA group 8 (1–22) years. The control group consisted of 31 men and 29 women with a mean age (range) of 39 (21–64) years. None of the patients was treated with steroids or immunosuppressive drugs. The mean non-steroidal anti-inflammatory drug (NSAID) consumption did not differ between the patient groups. Twenty of the patients with RA, randomly selected, were investigated by the Ritchie articular index.¹³

Fifteen patients with AS and 15 with RA were studied before and after three months' treatment with sulphasalazine (2–3 g/day). The clinical effect of treatment was evaluated by estimation of pain and stiffness on a visual analogue scale and NSAID consumption in patients with RA; in patients with AS the lumbar motion range (Schober's test) and presence of sacroiliac pain was also estimated. If improvement was found in more than half of the variables studied the patient was considered to be better.

LABORATORY INVESTIGATION

Elastase in ethylenediaminetetra-acetate plasma was measured in complex with α_1 proteinase inhibitor by means of an enzyme linked immunosorbent assay (ELISA).⁷ Venous blood samples were collected in the morning after an overnight fast. Plasma samples were separated within one to two hours by centrifugation. The patient and control plasma samples were stored at -70°C and later analysed in sequence. The intra-assay and day to day variation of the elastase measurements was $<10\%$. Serum levels of immunoglobulins and serum haptoglobin were measured by nephelometry at the Department of Clinical Chemistry, University Hospital, Uppsala. Reference values are given in Table 1. Erythrocyte sedimentation rate (ESR) was read after one hour by the Westergren method.

STATISTICAL ANALYSIS

Student's *t* test was used for analysis of normally distributed values. Non-parametric tests were used

for skewed distributed values. The means are presented as the arithmetic means (SD) or medians with first and third quartiles.

Results

The plasma elastase levels in patients with rheumatoid arthritis, ankylosing spondylitis, and in healthy controls are illustrated in Fig. 1. The values in patients with AS and in controls were normally distributed but as the values in the RA group showed a wide range, medians were calculated for

Fig. 1 Plasma levels of elastase, illustrated by a box-plot model, in healthy controls, patients with ankylosing spondylitis, and patients with rheumatoid arthritis. The medians and the first and third quartiles are illustrated. The minimum and maximum values are also indicated. For the patients with rheumatoid arthritis the individual elastase values are flagged.

Table 1 Laboratory data* of patients with ankylosing spondylitis (AS) and rheumatoid arthritis (RA)

Patients	n	ESR (mm/h)	Haptoglobin (g/l)	IgM (g/l)	IgG (g/l)	IgA (g/l)	PMN $\times 10^{-9}/l$
AS	42	27 (21)	2.9 (0.9)	1.8 (1.1)	12.6 (2.6)	3.2 (1.2)	5.2 (1.4)
RA	33	57 (28)	3.8 (0.6)	1.8 (0.7)	14.1 (5.2)	2.9 (1.5)	5.5 (1.6)
Reference values		<10	0.3–2.0	0.4–2.8	7–18	0.8–4.0	1.9–7.4

*Means (SD).

comparison. Patients with rheumatoid arthritis had significantly raised values when compared with controls ($p<0.001$), whereas patients with ankylosing spondylitis had normal values. No sex or age dependency was seen either in the patients or in the controls. Values for ESR, serum haptoglobin, immunoglobulins M, G, and A, and polymorphonuclear cell count are given in Table 1. The rheumatoid patients showed a slightly higher inflammatory activity, measured as ESR and haptoglobin, when compared with the AS group. No correlation was found between acute phase reaction, immunoglobulins, PMN cell count, and the plasma levels of elastase. In the 20 patients with rheumatoid arthritis evaluated by the Ritchie articular index a correlation was found between the Ritchie index and the plasma elastase values ($r=0.58$, $p<0.01$) as seen in Fig. 2. The patients with ankylosing spondylitis were almost completely free from peripheral joint involvement.

Fig. 2 Correlation between plasma elastase and Ritchie articular index in patients with rheumatoid arthritis ($n=20$).

Fifteen patients with ankylosing spondylitis and rheumatoid arthritis were studied before and after three months' treatment with sulphasalazine. Nine patients with ankylosing spondylitis out of 15 improved in three or more of the five clinical variables studied and in the group with rheumatoid arthritis 10 out of 15 improved clinically. In the group with spondylitis a reduction of the acute phase reaction measured as haptoglobin and IgA was seen (Table 2), but the plasma elastase levels remained unchanged. In the patients with rheumatoid arthritis a significant reduction of haptoglobin was seen ($p<0.001$) concomitant with a reduction ($p=0.08$) of the elastase levels (Fig. 3). The decrease in plasma elastase seemed to occur between three and six weeks.

Fig. 3 Reduction of plasma elastase (○) and serum haptoglobin (●) during treatment with sulphasalazine (2-3 g/day). Values are given as means (SEM). The shaded area represents the normal range for plasma elastase.

Table 2 Laboratory data* of patients with ankylosing spondylitis ($n=15$) before and after three months' treatment with sulphasalazine

	Haptoglobin (g/l)	IgA (g/l)	IgG (g/l)	IgM (g/l)	Elastase (µg/l)
Before	3.0 (0.3)	3.3 (0.3)	12.8 (0.7)	2.0 (0.4)	135 (13)
After	2.0 (0.3)†	2.9 (0.3)‡	11.6 (0.9)	1.6 (0.2)	144 (22)

*Means (SEM).
† $p<0.01$; ‡ $p<0.05$ when compared with pretreatment values.

Discussion

If this study we report normal levels of plasma elastase in patients with ankylosing spondylitis. This finding does not support previous reports of activated neutrophils in HLA-B27 associated arthritides.^{6,7} It is also in contrast with our previous findings of raised circulating levels of eosinophil cationic protein in patients with AS, which was interpreted as a sign of activation or enhanced turnover of eosinophils.⁸ The patients with rheumatoid arthritis investigated in this study, however, had significantly raised levels of elastase. The control group was matched with the patients with AS but not with the patients with RA. This fact can hardly influence the interpretation of the data as no age and sex dependency for elastase was found in patients or controls. Similar findings in RA have been reported in one previous study,¹⁴ but lowered elastase activity has also been reported.¹⁵ In the latter study a functional assay to determine elastase levels was used. This probably explains the discrepancies in results as most circulating elastase is bound in an inactive form.^{16,17} Our finding of raised elastase levels does not seem simply to reflect the acute phase reaction as no correlation was found with ESR or haptoglobin in either the patients with RA or in those with AS. Thus the slight difference in the degree of inflammatory activity in the two diseases can hardly explain the observed differences in elastase levels. The results may reflect differences in the turnover of granulocytes, which is greatly enhanced in RA.^{18,19} The possibility that the high elastase concentrations reported in inflamed synovial fluid²⁰ could contribute to the circulating levels is indirectly supported by the difference in peripheral joint involvement between the patients with RA and those with AS. The correlation found between plasma elastase and the Ritchie index in patients with RA further supports the suggestion that elastase from the inflamed joints influences the plasma levels.

The efficacy of sulphasalazine in the treatment of rheumatoid arthritis and ankylosing spondylitis is increasingly well recorded,²¹⁻²³ yet the exact mode of action of the drug in these diseases remains obscure. In vitro studies of granulocytes from patients with inflammatory bowel disease have shown an inhibitory influence of sulphasalazine and its components on migration, superoxide production, iodination mediated by myeloperoxidase, and cytotoxicity.²⁴ Inhibition of mast cell degranulation in rats by sulphasalazine has also been reported.²⁵ These findings suggest a possible direct influence of sulphasalazine on granulocyte activation in rheumatic diseases also. We have previously

reported a decrease of eosinophil cationic protein in patients with ankylosing spondylitis during treatment with sulphasalazine. This could be due either to a direct effect on the eosinophil or to an influence on the inflammatory processes that govern eosinophil activation. In this study no effect on the elastase levels was noted in the patients with AS during sulphasalazine treatment despite clinical improvement and reduction of the acute phase reaction. The patients with rheumatoid arthritis treated with the same drug also showed a reduction of their inflammatory activity but this paralleled a reduction of the plasma elastase levels. The lack of influence on plasma elastase levels in patients with AS and the late response in elastase reduction in patients with RA do not support the idea of direct influence of sulphasalazine on neutrophils. More likely the normalisation of plasma elastase reflects an indirect effect of sulphasalazine on neutrophils by influence on the mechanisms activating these cells in the circulation or in the inflamed synovial cavity, or in both.

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