

Inactivation of the elastase inhibitory activity of α_1 antitrypsin in fresh samples of synovial fluid from patients with rheumatoid arthritis*

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Abstract

The proteinase inhibitory ability of α_1 antitrypsin was measured in 23 samples of rheumatoid arthritis synovial fluid, eight osteoarthritic synovial fluids and nine normal control serum samples. For each sample a detailed kinetic analysis was performed with porcine pancreatic elastase as the target proteinase. Samples were stored for less than 24 hours at 4°C before analysis, which does not significantly alter the proportion of inactive α_1 antitrypsin. In rheumatoid synovial fluid the elastase inhibitory ability was disproportionately depressed relative to the immunochemically determined concentrations of α_1 antitrypsin.

α_1 antitrypsin is the main serine proteinase inhibitor in human plasma. One of its major physiological roles is to protect connective tissue from degradation by elastase.¹ Elastase, which is released in large amounts by neutrophils at sites of inflammation, has a wide substrate specificity, including collagen and proteoglycans. It has therefore been implicated in cartilage damage within the rheumatoid joint.¹

Normally free elastase is inhibited by α_1 antitrypsin owing to the rapid formation of a 1:1 complex, which slowly breaks down to yield elastase and inactive α_1 antitrypsin. In inflammatory conditions, however, stimulated neutrophils can inactivate the elastase inhibitory ability of α_1 antitrypsin both proteolytically, by releasing lysosomal enzymes, and oxidatively, by releasing reactive oxygen species.¹ Furthermore, in the arthritic joint the cartilage surface itself may interfere with the interaction between α_1 antitrypsin and elastase.² It was suggested² that because α_1 antitrypsin carries a net negative charge at neutral pH it has a relatively poor ability to diffuse into the negatively charged cartilage. In contrast, elastase is positively charged. In rheumatoid arthritis the combination of these effects might permit elastase to degrade proteins within the cartilage matrix, even though α_1 antitrypsin is normally present in molar excess over elastase in the synovial fluid.

Previous studies of synovial fluid samples from patients with rheumatoid arthritis concluded that α_1 antitrypsin is both cleaved and oxidised,³ leading to substantial inactivation.⁴⁻⁶ These studies, however, were conducted on samples stored frozen at -10°C, -20°C, or at an unspecified temperature. Such storage of human extracellular fluids leads to both oxidation and proteolysis of constituent proteins, resulting in a loss of functional activity.⁷⁻⁹ This

suggests that earlier studies of α_1 antitrypsin in rheumatoid synovial fluid³⁻⁶ might have been affected by changes induced by storage. Furthermore, in the previous studies the functional activity of α_1 antitrypsin was assayed using a single active site titration of trypsin for each sample. To consider these problems we reassessed the functional activity of α_1 antitrypsin in fresh samples, performing a detailed kinetic analysis of inhibitory activity towards porcine pancreatic elastase.

Patients and methods

Synovial fluids were aspirated from the knees of patients with rheumatoid arthritis and osteoarthritis (American Rheumatism Association criteria) for genuine therapeutic purposes. Serum samples were obtained from normal healthy control subjects. Samples were centrifuged at 2400 g for 10 minutes, stored at 4°C, and analysed within 24 hours of collection. These conditions of storage produced no significant change in the proportion of inactive α_1 antitrypsin.

Inactivation of α_1 antitrypsin was assessed, firstly, by an immunochemical assay, and, secondly, by its functional (elastase inhibitory) activity. The immunochemical determination of α_1 antitrypsin was carried out by rocket immunoelectrophoresis, permitting calculation of the molar concentration of α_1 antitrypsin in each fluid. The antibody to α_1 antitrypsin (Sigma, Dorset, UK) determines all forms of α_1 antitrypsin—free, bound to proteinase, and released and inactivated. The ability of each fluid to inhibit the cleavage of the synthetic substrate *N*-succinyl-Ala-Ala-Ala-*p*-nitroanilide (Sigma) by porcine pancreatic elastase (Sigma) was determined by a kinetic spectrophotometric method.¹⁰ The inhibitory activity of each fluid was determined at five different sample dilutions. The values obtained from the immunochemical and kinetic assays were combined to give the specific elastase inhibitory activity of α_1 antitrypsin in each fluid. This was defined as the number of moles of α_1 antitrypsin required to inhibit one mole of elastase completely. For convenience, the specific elastase inhibitory activity of normal serum was defined as one mole of α_1 antitrypsin required per mole of elastase.

Statistical analysis of results was by Student's *t* test and by Spearman's rank correlation coefficient.

Results and discussion

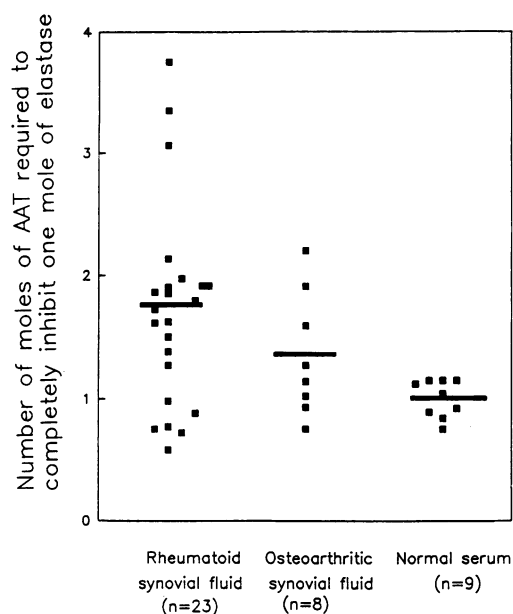
Normal serum contained 3.74 (1.50) (mean (SD)) mg/ml α_1 antitrypsin. As expected, the

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Specific elastase inhibitory activity, expressed as the number of moles of α_1 antitrypsin required to inhibit one mole of elastase completely, in fresh samples of rheumatoid synovial fluid, osteoarthritic synovial fluid, and normal control serum samples. The bar (—) indicates the mean value within each group.

immunochemically determined concentration of α_1 antitrypsin was significantly raised in rheumatoid synovial fluid (4.29 (1.9) mg/ml) compared with osteoarthritic synovial fluid (2.17 (0.81) mg/ml; $p < 0.001$). The number of moles of α_1 antitrypsin required to inhibit one mole of elastase (figure) was also significantly higher in fresh rheumatoid synovial fluid than in fresh normal control serum samples ($p < 0.001$), indicating a lower specific elastase inhibitory activity equivalent to 41% inactivation of α_1 antitrypsin. The α_1 antitrypsin in osteoarthritic synovial fluid also seemed to be partially inactivated (25%), though this was not statistically significant.

There was a relatively large scatter in the specific elastase inhibitory activity of the rheumatoid synovial fluid samples (figure). Specific elastase inhibitory activity was correlated with α_1 antitrypsin concentration for each of the three groups of samples. For the rheumatoid synovial fluid group Spearman's rank correlation coefficient (r_s) was 0.46 ($p < 0.05$); there was no significant correlation in either the osteoarthritic synovial fluid group ($r_s = 0.60$) or the normal serum group ($r_s = 0.06$). The significant correlation in the rheumatoid synovial fluids implies that a more severe inflammatory response will result in both a higher concentration of immunoreactive α_1 antitrypsin and a greater inactivation of α_1 antitrypsin by reactive oxygen species and lysosomal proteinases released by infiltrating neutrophils.

Our results obtained with fresh rheumatoid synovial fluid support the earlier suggestions³⁻⁶ that substantial inactivation of α_1 antitrypsin occurs in vivo. The results reported here are

also consistent with our earlier observation that a substantial proportion of α_1 antitrypsin is present in a cleaved form in fresh rheumatoid synovial fluid.¹¹ As storage induces the inactivation of α_1 antitrypsin it might have been expected that the concentrations of inactive α_1 antitrypsin in the present study would be lower than those found previously for stored samples (15%–30%),⁴⁻⁶ but we found a higher proportion of inactive α_1 antitrypsin in rheumatoid synovial fluid.

This observation may be explained by further important differences between this study and earlier ones. Firstly, the target proteinase used in our assay of α_1 antitrypsin activity was porcine pancreatic elastase, whereas other workers used trypsin. Secondly, we used several dilutions of the fluids containing α_1 antitrypsin to perform a detailed kinetic analysis, whereas the trypsin assay was by a single active site titration. Finally, this difference might be attributable to a varying severity of inflammation in the patient groups studied because, as mentioned above, our results imply that the severity of inflammation correlates with the extent of α_1 antitrypsin inactivation.

We conclude that substantial inactivation of α_1 antitrypsin occurs within the rheumatoid joint which, in combination with the negative charge repulsion between α_1 antitrypsin and cartilage, could result in discrete areas of α_1 antitrypsin deficiency and hence elastolytic activity leading to cartilage damage.

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