α_1 -Antichymotrypsin in Rheumatoid Arthritis*

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KOSAKA, S. and TAZAWA, M. *α_1 -Antichymotrypsin in Rheumatoid Arthritis.* Tohoku J. exp. Med., 1976, 119 (4), 369-375 — Serum α_1 -antichymotrypsin (α_1 -ACT) of the patient with rheumatoid arthritis was studied by means of single radial immunodiffusion method. There was a significant elevation of the α_1 -ACT concentration in the patients with rheumatoid arthritis, and positive relationships were observed between the concentrations of α_1 -ACT and of other glycoproteins such as α_1 -acid glycoprotein and α_1 -antitrypsin in individual patients, and between C-reactive protein (CRP) rates and the α_1 -ACT concentrations in individual specimens. These facts suggest that α_1 -ACT belongs to a group of acute phase proteins like α_1 -antitrypsin or CRP. An inverse proportional correlation was revealed between α_1 -ACT and fibrinolytic activity. No influences were observed on the α_1 -ACT concentration, activity index or articular index by the oral administration of α -chymotrypsin in patients with rheumatoid arthritis. ——— α_1 -antichymotrypsin; rheumatoid arthritis; proteinase inhibitor; acute phase protein

In the present decade, considerable progress has been made toward the purification and characterization of the individual components of human serum comprising the total proteinase-inhibiting capacity. The following picture has emerged from these investigations: There are now five known antiproteinases in human serum capable of inhibiting trypsin, chymotrypsin, elastase and proteinases from granulocytes. They are α_1 -antitrypsin which is responsible for approximately 90 percent of the total inhibiting capacity (Schultze et al. 1962), α_2 -macroglobulin, α_2 -antitrypsin, the inter- α -trypsin inhibitor and α_1 X-glycoprotein (Schwick et al. 1966).

These proteinase inhibitors are very important due to the fact that their inborn deficiencies may lead characteristic diseases. Hereditary deficiency of α_1 -antitrypsin is associated with chronic obstructive pulmonary disease (Laurell and Eriksson 1963; Eriksson 1965) and severe infantile cirrhosis (Sharp et al. 1969). Kosaka and Kitabatake (1970) previously reported the significant elevations of α_1 -antitrypsin and α_1 -acid glycoprotein in the sera of patients with rheumatoid arthritis.

The α_1 X-glycoprotein was first found by Schultze et al. (1962) who detected an unknown band of precipitate which belonged to the α_1 -globulin region in

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human serum in the immunoelectrophoresis, and isolated this glycoprotein. A few years later Heimburger and Haupt (1965) confirmed that the α_1 X-glycoprotein was a protease inhibitor which showed a high specificity for chymotrypsin. Therefore, this glycoprotein is now also called α_1 -antichymotrypsin (α_1 -ACT).

Nevertheless, literatures dealing with other biological functions as well as the pathological significance of α_1 -ACT are still scanty.

In the present studies, the concentrations of α_1 -ACT were determined and were compared with the levels of other two glycoproteins, C-reactive protein (CRP) and fibrinolytic activity in the sera of patients with rheumatoid arthritis. The influence of administration of α -chymotrypsin on the α_1 -ACT concentrations, activity index and articular index in these patients were also examined.

MATERIAL AND METHODS

Determination of the α_1 -ACT concentration in serum was carried out by the single radial immunodiffusion method as follows (Mancini et al. 1965): 10 ml of warm 1.25% agar gel were mixed with 0.25 ml of anti-antichymotrypsin rabbit serum (Behringwerke/Marburg) and poured on a Partigen plate to give a 2-mm thick layer. After congelation circular wells with a diameter of 2 mm were punched out. Exactly 2 μ l of test serum were introduced into each of the wells by means of a micropipette. After the plate was incubated for 48 hr at room temperature, the formation of a visible ring of precipitate around the wells was examined. The α_1 -ACT concentration was estimated from the calibration curve which was designed for a series of dilution of standard human serum (Behringwerke) in reference to the variations of the ring diameter.

Serum concentrations of α_1 -antitrypsin and α_1 -acid glycoprotein were simultaneously measured with Partigen plate kit (Behringwerke) according to the procedure of single radial immunodiffusion method. In order to quantify the CRP, commercially available anti-CRP prepared from sheep and goat (Hyland Lab./Los Angeles) was used. Fibrinolytic activity was measured by the fibrin plate method of Astrup and Müllertz (1952).

RESULTS

The α_1 -ACT concentrations in sera from healthy control persons and from patients with rheumatoid arthritis

The data are summarized in Table 1. Serum samples of controls were taken from 54 males and 26 females at the age of 20 to 25. The α_1 -ACT values of these

TABLE 1. α_1 -Antichymotrypsin concentrations in

Patients	Sex	Number of cases	α_1 -Antichymotrypsin	
			31 ~ 40	41 ~ 50
Rheumatoid arthritis	Female	41		8
	Male	6		1
	Total	47		9
Controls	Female	26	10	14
	Male	54	22	27
	Total	80	32	41

cases were in the range between 31 and 54% and the values seemed to represent the normal range. The average values for males and females were 41% and 42%, respectively, suggesting no sex difference in the average value.

In 47 cases of classical or definite rheumatoid arthritis which were composed of 6 males and 41 females, the α_1 -ACT concentrations were between 41 and 96% (61% on the average). This indicates that the values were significantly increased in patients with rheumatoid arthritis as compared with the normal healthy persons ($p < 0.001$).

Relationship between α_1 -ACT and other glycoproteins

It is ascertained that both α_1 -antitrypsin and α_1 -acid glycoprotein are glycoproteins and moreover belong to the same α -globulin fraction as the α_1 -ACT (Schultze and Heremans 1966). There was a high correlation between α_1 -ACT and α_1 -antitrypsin (Fig. 1) as well as the α_1 -acid glycoprotein (Fig. 2), showing 0.38 of coefficient of correlation for α_1 -antitrypsin and 0.67 for α_1 -acid glycoprotein. A higher correlation was observed between α_1 -ACT and α_1 -acid glycoprotein than

Fig. 1. Correlation between α_1 -antichymotrypsin and α_1 -antitrypsin.

patients with rheumatoid arthritis and healthy controls

concentrations (%)					
51~60	61~70	71~80	81~90	91~100	Total means
15	10	7	1		58
	1	1	2	1	76
15	11	8	3	1	61
					42
2					41
5					41.5
7

Fig. 2. Correlation between α_1 -antichymotrypsin and α_1 -acid glycoprotein.

between the former and α_1 -antitrypsin.

Relationship between α_1 -ACT and CRP

In rheumatoid arthritis, CRP is present in practically all patients with clinical evidence of disease activity. It tends to parallel the sedimentation rate closely (Ziff and Baum 1966). In the present study, a proportional correlation was found between the α_1 -ACT levels and degrees of the CRP rates (Fig. 3). On the basis of this finding, it seems that α_1 -ACT has also some relation with the activity of the disease in the patients.

Fig. 3. Correlation between α_1 -antichymotrypsin and C-reactive protein.

Relationship between α_1 -ACT and fibrinolytic activity

An inverse relationship was indicated to be present between α_1 -ACT and fibrinolytic activity (-0.24 of coefficient of correlation) as can be seen in Fig. 4. This finding may suggest that α_1 -ACT reacts possibly antagonistically against fibrinolytic enzyme.

Fig. 4. Correlation between α_1 -antichymotrypsin and fibrinolytic activity.

Changes in the α_1 -ACT levels, activity indices and articular indices after oral administration of proteinase in the patients

Twelve patients were orally given α -chymotrypsin as proteinase, 600 ch. units, daily for 6-8 weeks. The α_1 -ACT concentrations were determined every 2 weeks but the concentration change was quite variable during these periods of examination; 2 showed a marked increase, 2 moderate increase, 3 no-change and 5 decrease. At the same time, the activity indices (Lansbury 1966), whose factors consist of duration of morning stiffness, grip strength, sedimentation rate and speed of gait, were also examined and the indices also showed considerable variations and had no correlation with the concentration of α_1 -ACT in each individual. The results of examination of articular indices, estimated from the total amount of active joint inflammation in which all peripheral joints were examined for tenderness on pressure or pain on passive motion, were also variable in the same manner as for the activity indices.

DISCUSSION

The presence of high concentrations of α_1 -ACT as proteinase inhibitor in the sera of the patients with rheumatoid arthritis is of considerable interest. The finding in the present study agrees with the earlier observations by Brackertz and others (1975) who showed a significant rise in α_1 -ACT, α_1 -antitrypsin and inter-

α -trypsin inhibitor in serum as well as in synovial fluid of patients with rheumatoid arthritis. They found that the concentrations of these proteinases were also significantly higher as compared with those of the patients with osteoarthritis without inflammatory process. This difference is in agreement with the known fact that α_1 -antitrypsin and α_1 -ACT (Kueppers 1968; Aronsen et al. 1972) belong to the group of acute phase proteins that respond to inflammatory stimuli with a substantial rise in concentration. This finding could also be explained by the fact that the α_1 -ACT values correlated well with CRP rates reported in this paper. On the one hand, Fricke and Rohne (1971) reported that levels of α_1 -acid glycoprotein in the sera were a better indicator of the activity of rheumatoid arthritis than sedimentation rates, since the concentration of this glycoprotein increased in chronic inflammation and was directly proportional to the activity of the disease. The α_1 -acid glycoprotein may have a character of acute phase protein such as α_1 -ACT, because an intimate correlation was noted between these glycoproteins. It seems that the presence of protease inhibitors at the site of inflammation, first shown by Opie and Barker (1907), is important in limiting the destructive activity of proteases liberated from polymorphonuclear leucocytes and other necrotizing tissues. Two such enzymes, at least, have been isolated and characterized (Janoff and Scherer 1968; Lazarus et al. 1968).

Some of the proteinase inhibitors constitute a part of the control mechanism of fibrinolysis and coagulation, and of the kinin and complement system. It is well known that these systems can release biologically active peptides that have potent effects on blood vessels and on the activity of leucocytes. Anti-proteases are probably involved in the protection of tissues against proteolytic enzymes which are released from leucocytes and other cells in various pathological states by limiting their proteolytic activity. It is widely held that proteolytic enzymes in rheumatoid arthritis are responsible for much of the tissue damage in affected joints.

Therefore, a change of the serum α_1 -ACT levels after administration of the protease such as α -chymotrypsin against the patients with rheumatoid arthritis is of interest. No consistent variation, however, was observed in the α_1 -ACT levels or in the activity indices and the articular indices. There is a possibility that the protease given orally might not be absorbed sufficiently through intestine. It is still necessary to continue further investigation on this aspect.

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