Serum α -thiol protease inhibitor concentrations in health and disease

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SUMMARY Serum α -thiol protease inhibitor (α -TPI) concentration was assayed by radial immunodiffusion in normal subjects, pregnant women, and in a wide variety of diseases. The normal concentration (448 (SD 75) mg/l) increased significantly ($p < 0.001$) in pregnancy to 575 (89) mg/l, and in prostatic cancer treated by oestrogens to 666 (87) mg/l. Inconsistent changes were observed in inflammatory and malignant disease and in liver disease. A temporary pronounced fall of α -TPI was seen after burn injury, and a sustained fall after bone marrow transplantation. Crossed immunoelectrophoresis showed that the serum α -TPI occurred in two forms, with α_2 and α_1 electrophoretic mobilities. A heavy demand on this antiprotease may result in suppression of the α_1 form.

The control of proteolysis is of fundamental importance in maintaining normal tissue function, and the antiproteases in the blood provide a major system to oppose the activity of a wide diversity of proteolytic enzymes. The most widely studied in disease are the serine antiproteases, such as α_1 -antitrypsin (α_1 -AT), α_1 -antichymotrypsin (α_1 -ACT), and α_2 -macroglobulin, as well as the antiproteases that regulate the clotting cascades and fibrinolysis.¹

Fundamental studies of cellular lysosomal enzymes have identified families of proteases with specificities other than serine. Lysosomal cysteine (thiol) proteinases have a key role in the catabolism of intracellular protein. Cathepsin B, H, and L are the most studied enzymes of this group. Several extracellular inhibitors specific for cysteine proteases have been discovered, and in addition to these specific antiproteases, haptoglobin, α_2 -macroglobulin, IgG and antithrombin III also inhibit cysteine proteinases.²

Serum α -thiol protease inhibitor (α -TPI) was first described in 1977 by Sasaki *et al*³ as a protein with an α_2 mobility and a molecular weight of 90 000 daltons. In 1979 Ryley⁴ and Jarvinen⁵ both reported that TPI isolated from human serum was not a single component but existed in two immunologically identical forms. The main component had a molecular weight of 90 000 daltons and ran in the α_2 region and the other had an α_1 mobility and a molecular size of 170 000–175 000 daltons. Further heterogeneity of

TPI was described recently by Sasaki *et al*,⁶ who found three antigenically identical forms, two of which ran in the α_2 region. Antigenically active fragments of α -TPI of molecular weight 70 000 and 22 500 daltons have been found in normal human urine.⁷ Other inhibitors of cysteine proteinases, with lower molecular weights (11 000–16 000 daltons) have also been reported,⁸ an example of which is cystatin, also called γ -trace protein and post- γ -globulin.⁹

Alpha-TPI inhibits thiol proteases but does not inhibit serine proteases, such as trypsin, chymotrypsin, or elastase. The report by Ryley⁴ found that the normal serum concentration of α -TPI was 420 mg/l, with only 240 mg/l in umbilical cord serum. The behaviour of this protein in disease has not been examined, and in this paper we therefore looked at its concentration in normal controls, pregnancy, and a variety of inflammatory and malignant diseases, as well as in advanced renal and liver disease. Alpha-TPI was also measured in patients after severe burn injury and after bone marrow transplantation.

Material and methods

Serum was obtained from normal blood donors, pregnant women, and a variety of patients, chosen to examine the behaviour of α -TPI in malignant and non-malignant diseases. The samples were stored at -20°C before their analysis.

Serum α -TPI concentrations were measured by radial immunodiffusion,¹⁰ using antisera and standards provided by Behringwerke AG, Marburg,

Lahn, West Germany. Measurements of serum C-reactive protein (CRP), α_1 -ACT, α_1 -acid glycoprotein (AGP), and steroid binding globulin (SP2) were also made on some selected patient groups by single radial immunodiffusion. The α_1 and α_2 forms of TPI were identified by crossed immunoelectrophoresis. Microheterogeneity of the glycan chains was examined by immunoaffinoelectrophoresis using concanavalin A in the first dimension gel, as previously described by Wells *et al.*¹¹

The statistical correlations were examined by the Wilcoxon's rank test, which is not influenced by a skewed distribution of the analytes.

Results

The interassay coefficient of variation (CV) measured on a control serum run in duplicate over 27 α -TPI radial immunodiffusion plates was 9.8%.

Table 1 shows the concentrations of α -TPI found in normal subjects, pregnant women, and in benign and malignant diseases. There was a significant increase ($p < 0.001$) in the α -TPI concentration in pregnant women (> 6 months) and in men with prostatic cancer receiving treatment with stilboestrol.

Of the inflammatory diseases studied, only the patients with pneumonia and rheumatoid arthritis showed a significant reduction ($p < 0.001$) in the mean α -TPI concentration. The α -TPI concentrations in pneumonia and Crohn's disease showed no correlation with the CRP, α_1 -ACT, or α_1 AGP values,

Table 1 Concentration of serum α -TPI in normal subjects, pregnancy, and various diseases

Condition	Total no	Mean (mg/l)	(SD) (mg/l)
Normals:			
Sex unknown	40	466	81
Men	23	426	53
Women	24	439	76
Pregnancy:			
< 6 months	40	446	84
> 6 months	38	575	89
Inflammatory disease:			
Pneumonia	40	336	70
Crohn's disease	50	470	102
Rheumatoid arthritis	40	369	59
Other benign diseases:			
Renal disease	40	463	69
Liver disease	38	398	110
Benign prostatic hyperplasia	20	442	61
Malignant disease:			
Colorectal cancer	43	421	93
Lung cancer	19	384	52
Myelomatosis	39	378	63
Breast cancer (stages I and II)	28	414	55
Breast cancer (stage III)	12	506	109
Prostate cancer (no treatment)	30	429	50
Prostate cancer (on oestrogens)	37	666	87
Acute leukaemia	37	351	93

indicating that TPI does not behave as an acute phase protein.

In renal disease there was no difference between the mean of those patients with a serum creatinine of $< 140 \mu\text{mol/l}$ (α -TPI = 455 (49) mg/l) and those with a creatinine of $> 140 \mu\text{mol/l}$ (α -TPI = 471 (86) mg/l).

The concentrations of α -TPI in liver disease showed a considerable variation, as shown by the large standard deviation. There was no general tendency for the α -TPI values to be related to a particular form, but the lowest values were observed in portal hypertension without cirrhosis. There was no relation between the α -TPI concentrations in liver disease and the SP₂ values, which ranged from normal, < 12 , to 30 mg/l.

In malignant disease the α -TPI measurements were generally in the normal range, but a significant ($p < 0.001$) reduction in values was seen in the groups with lung cancer, myelomatosis, and acute leukaemia.

The lowest values were observed immediately following severe burn injury and in patients who had undergone bone marrow transplantation. Figs 1 and 2 show that the α -TPI concentrations recovered rapidly after burn injury but remained low for several weeks after bone marrow transplantation. Table 2 shows the mean concentrations of α -TPI in 26 patients before and after bone marrow transplantation for either acute leukaemia (myeloid and lymphocytic) or chronic granulocytic leukaemia.

In several isolated serum samples from patients with cancer and pregnant women no precipitin ring on radial immunodiffusion was visible after staining. Prolonged standing at room temperature or repeated freezing and thawing did not affect the formation of precipitin rings from 10 normal serum samples. If the

Fig 1 Alpha-TPI concentration after severe burn injury. (Case 1 = 22%, case 2 = 57% body surface area burn.)

Fig 2 Alpha-TPI concentration after bone marrow transplantation. Cases 3 and 4 = chronic granulocytic leukaemia; case 5 = acute myeloblastic leukaemia.

same samples were incubated for three days at 2°C at a 1/10 dilution with a pregnancy sample which gave no precipitin ring, however, the α -TPI was no longer measurable by radial immunodiffusion.

VARIATION IN THE α_1 AND α_2 FORMS

Crossed immunoelectrophoresis clearly showed that the greater part of TPI has an α_2 mobility, which corresponds to the 90k monomer and the α_1 form of the 170k dimer is the smaller component (fig 3). Two peaks were observed in all but one of 16 normal subjects, who exhibited only the α_2 peak. In both normal and diseased patients a double arc could often be seen (arrows fig 3) above the area of both peaks with a discontinuity in the precipitin line suggestive of further microheterogeneity within this protein.

In inflammatory disease the α -TPI peak was considerably reduced and was only represented by a trace in the burns sera. A similar result was obtained after bone marrow transplantation. In pregnancy the peak heights of both the α_1 and α_2 forms were increased.

Crossed immunoaffinoelectrophoresis with concanavalin A in the first dimension gel produced only a weak retardation of the α_1 and α_2 peaks. In pregnancy

Table 2 Mean (SD) α -TPI concentration (mg/l) after bone marrow transplantation

Before transplantation	After transplantation			
	7 days	28 days	90 days	
4 days				
366 (87)	319 (72)	348 (91)	386 (95)	
(n = 26)	(n = 26)	(n = 26)	(n = 15)*	

*There were no late follow up samples in six children. Five patients died between 28 and 90 days, and the mean α -TPI value in these five at 28 days was 265 (77) mg/l.

there was no evidence of any accentuation of the diversity of glycan branching, as the peaks with and without concanavalin A in the first dimension gel had a similar appearance.

Discussion

The activity of an antiprotease in the blood is influenced by three main factors: the rate of production and release from the site of origin; the half life of the protein in the circulation; and the rate of removal as inhibitor-protease complexes. The efficiency of the hepatic lectin to recognise and capture aged molecules will depend on the lectin recognising terminal galactose groups on the oligosaccharide chains that are exposed after the loss of sialic acid. Alpha-TPI contains 13% carbohydrate.⁴

The results of the present studies can only hint at some of the factors that may be operating to influence the concentrations of α -TPI in disease. The mean α -TPI value in normal subjects (420 mg/l) agrees well with that reported by Ryley.⁴ There is no difference in the values seen in healthy men and women, but there is a progressive rise in pregnancy, and high values can be induced in men by oestrogens. In this respect α -TPI responds to oestrogens in a similar fashion to α_1 -AT and caeruloplasmin, both of which are increased in pregnancy, but unlike these acute phase reactants there is no shift in the distribution of the oligosaccharides to favour the synthesis of triantennary or tetra-antennary chains.¹² The lack of significant binding of α -TPI to concanavalin A suggests that the glycans are predominantly of the triantennary form, as it is the biantennary forms that bind to concanavalin A

Fig 3 Crossed immunoelectrophoresis of serum samples (5 µl) against antiserum to α-TPI (1 µl/cm²) in second dimension.

Number sample	α-TPI (mg/l)	Number sample	α-TPI (mg/l)
a Normal	509	e Prostatic cancer (no treatment)	450
b Crohn's disease (high acute phase proteins)	418	f Prostatic cancer (given stilboestrol)	736
c 50 hours after severe burn injury	304	g Six days after bone marrow transplantation	217
d Pregnancy 7½ months	578	h Stage III breast cancer	466

and produce the microheterogeneity of the protein that is seen in the crossed immunoaffino-electrophoresis of α_1 -AGP and α_1 -AT.¹² In chronic liver disease it could be argued there are two opposing factors; one being the tendency for the concentrations of oestrogen to rise, as indicated by the induction of high values of steroid binding globulin (SP₂), the other being a fall in the number of hepatocytes and the probable decrease in liver lectin. Clearly, the absence of a correlation between SP₂ and α -TPI values showed that any oestrogen effect on α -TPI production is weak in liver disease.

In some diseases, such as pneumonia where the acute phase reaction is well developed, the low serum α -TPI values may be due to an increased consumption of this inhibitor by proteases released from inflamed cells. This may be an explanation for the low values found after severe burn injury and following bone marrow transplantation. The very low α -TPI concentrations seen immediately after burn injury may, however, be due in part to loss of body fluids. Alpha-TPI was shown to be present at about 75% of the serum concentration in three samples of blister fluid. The values of α -TPI in advanced cancers are variable and unrelated to tumour mass, which precludes it being a useful analyte to monitor or assess disease activity in patients with cancer.

The change in the relative concentrations of the α_2 and α_1 forms seem to occur when there is a demand on the system, but these were not affected by the hyperproduction induced by oestrogens. It cannot be determined whether this change in these two forms is due to the preferential use of the dimer for anti-protease activity and its removal as a complex, or whether the dimerisation is inhibited. The loss of the dimeric form was most noticeable shortly after severe burn injury when it almost disappeared.

The lack of change of the serum values in renal failure of neutral cysteine proteinase inhibitor, which is probably identical with α -TPI, was also observed by Hopsu-Havu *et al.*¹³ This is in sharp contrast to cystatin (molecular weight 13 000 daltons), which rises progressively as the glomerular filtration rate falls.¹³ The half lives of the two principal molecular size forms of α -TPI will differ considerably, as like the larger glycoproteins, they are in the range of four to eight days, whereas low molecular weight proteins, such as β_2 -microglobulin (molecular weight 11 300 daltons), have half lives of about one hour.¹⁴

This survey has raised several questions about the factors controlling the serum concentrations of this form of antiprotease and has shown several conditions in which prolonged depletion of α -TPI may

occur. The induction of α -TPI by oestrogens, however, provides yet another example of the widespread changes that occur in the composition of anti-proteases and certain acute phase reactant proteins in pregnancy, while the advantage of this change is still a matter for conjecture.

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