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Decreased Plasma α_1-antiproteinase Inhibitor Activity in Idiopathic Nephrotic Syndrome

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Summary. **Recent reports on a possible role for neutral proteinase(s), mainly serine proteinase(s), in the development of the nephrotic syndrome have prompted us to investigate the proteinase inhibitor activity of plasma α_1-proteinase inhibitor (α_1-PI, formerly referred to α_1-antitrypsin), which regulates a wide range of serine proteinases, including elastase. The concentration of plasma α_1-PI was significantly lower in idiopathic nephrotic syndrome (INS) both during relapse and in remission than in either chance proteinuria and/or haematuria or normal controls. The anti-elastase activity, one of the serine proteinase inhibitor effects of α_1-PI, was also decreased both during relapse and remission of INS. In contrast, the anti-trypsin activity was decreased upon relapse but normal in remission. The concentration of plasma α_1-PI correlated with the plasma anti-elastase activity, but not with the anti-trypsin activity. The presence of decreased levels of both plasma α_1-PI inhibitor concentration and anti-elastase activity, not only during relapse but also in remission of INS, was difficult to explain in the present study, but may be a predisposing factor for the development of proteinuria and resultant nephrotic syndrome.**

INTRODUCTION

Recently, several studies have demonstrated a possible role for certain neutral proteinases, mainly serine proteinase(s), in reducing anionic sites on the glomerular basement membrane and in the development of proteinuria.[1-4] In normal subjects, proteinase is inactivated by a requisite level of various proteinase inhibitors. However, in subjects with decreased plasma anti-proteinase activities, a subsequent proteolytic injury by proteinases would be difficult to control.

Of the seven major plasma proteinase inhibitors, α_1-proteinase inhibitor (α_1-PI) is present in the greatest quantities in the molars,[5] and is capable of inhibiting neutral proteinases including serine proteinases. At a clinical level, plasma α_1-PI is considered to play a role in the regulation of haemostasis as one of the thrombin inhibitors such as α_2-macroglobulin and anti-thrombin III, and has also been viewed as one of the acute phase reactants. α_1-PI inhibits various proteinases including serine proteinases such as pancreatic trypsin (the name is derived from this activity), elastase, cathepsin G, chymotrypsin, and thrombin.[5] However, the anti-trypsin effect of α_1-PI is no longer viewed as its major function, because the half-time of the association rate with trypsin is much longer than that with either elastase or cathepsin G.[5]

Although the plasma concentration of α_1-PI has been reported to decrease in idiopathic nephrotic syndrome (INS),[6-8] the pathological and causative significances have been discussed mainly in reference to the resulting coagulation abnormality. However, on the basis of serial observations which demonstrate a possible role for neutral proteinase in initiating nephrotic syndrome, the proteinase inhibitor activity of the plasma α_1-PI on neutral proteinases needs to be investigated in relation to the pathological state of INS. The aim of this study is to clarify both the quantitative and functional changes of plasma α_1-PI as an anti-serine proteinase inhibitor in patients with INS.

SUBJECTS AND METHODS

Twenty-two pediatric patients with INS including 13 patients during relapse, 20 patients with chance proteinuria and/or hematuria (CPH), and 10 normal control subjects were included in this study. Clinical

Table 1. Clincal characterstics of the subjects

	INS relapse	INS remission	CPH
number of patients	13	22	20
age (years)	6-24	6-24	4-18
sex (male, female)	18, 4	9, 4	6, 14
urinary protein (mg/dl)	210-1,250*	4-18	6-210
urinary RBC counts	<5/HPF	<5/HPF	3-5/HPF~massive
serum creatinine (mg/dl)	0.5-1.2	0.5-1.1	0.6-1.2
blood urea nitrogen (mg/dl)	8-24	6-19	8-23
treatment	prednisolon initiallly 60 mg/m^2 later tapered	supportive	dipyridamole trimetazidine HCl indomethacine cyclophosphamide or combination of any of the above drugs

INS: idiopathic nephrotic syndrome
CPH: patients in whom proteinuria and/or hematuria were found on routine school screening for renal diseases in Niigata; they are known to have various types of glomerulonephritis.
RBC: red blood cells
HPF: high-power field (×400) on light microscope

characteristics of the patients are shown in Table 1.

Concentrations (mg/dl) of plasma α_1-PI were determined by a single radial immunodiffusion technique. The two proteinase inhibitor activities of α_1-PI were measured, as follows, by the method of Beaty[9] with some modification.

Anti-trypsin activity: A 30 μl sample of plasma was diluted with 300 μl of 0.001 M Tris-HCl buffer at pH 8.0 in a test tube. 100 μl of diluted plasma was added to a test tube containing 100 μl of trypsin solution (Porcine trypsin, Sigma, 5 mg/ml in 0.01 M Tris-HCl buffer at pH 8.0) and allowed to incubate at room temperature for 3 min. To the reaction mixtures, 3.0 ml of substrate solution (0.58 mM Benzoyl L-arginine ethyl ester HCl, Sigma) was added and incubated at 25℃. The changes in optical density at 253 nm (OD_{253}) were read with standard trypsin solutions and ΔOD_{253}/min was calculated. Plasma anti-trypsin activity was expressed as units of trypsin inhibited by plasma per min (U/ml).

Anti-elastase activity: A sample of 5 μl of plasma was added to a test tube containing 1.1 ml of elastase solution (Porcine pancreatic elastase, Sigma, 0.3 mg in 5.0 ml of 0.2 M Tris-HCl buffer at pH 8.0) and allowed to incubate at room temperature for 3 min. To the reaction mixture, 2.0 ml of substrate solution (succinyl-L-alanyl-L-alanyl-p-nitroanillide, 45.0 mg in 20.0 ml of Tris-HCl buffer) was added and incubated at 40℃ to accelerate the reaction. The changes in optical density at 410 nm were read with standard elastase solutions. Plasma anti-elastase activity was expressed as units of elastase inhibited by 1.0 ml of plasma per min (U/ml).

Statistical analysis of the mean differences was carried out using Student's t-test.

RESULTS

1. Concentrations of plasma α_1-PI (Fig. 1)

The protein concentrations of plasma α_1-PI were 189.5±31.7 mg/dl in INS during relapse, 175.1±43.4 mg/dl in INS in remission, 212.9±23.7 mg/dl in CPH, and 216.7±24.9 mg/dl in control subjects. The values in INS during both relapse and remission were significantly lower than those in CPH and controls ($p<0.02$, $p<0.01$ respectively). There was no significant difference between CPH and control values.

2. Plasma anti-trypsin activity (Fig. 2)

Plasma anti-trypsin activity of α_1-PI was 321.0±156.3 U/ml in INS during relapse, 410.2±127.0 U/ml in INS in remission, 447.1±96.8 U/ml CPH, and 495.1 U/ml in controls. The mean value in INS during relapse was significantly lower than that in CPH ($p<0.01$) and in controls ($p<0.02$). No significant difference was found between the values of INS during relapse and remission.

3. Plasma anti-elastase activity (Fig. 3)

Anti-elastase activity of α_1-PI was 1211.7±377.1 U/

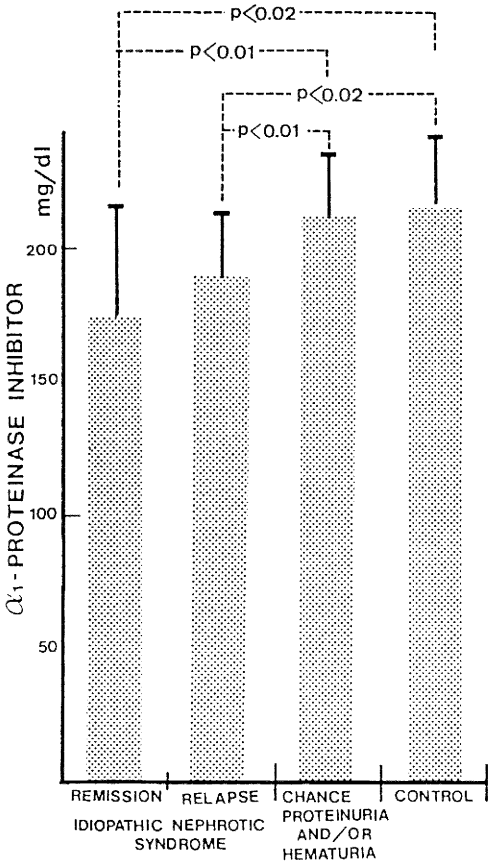

Fig 1. Plasma α1-proteinase inhibitor concentrations.

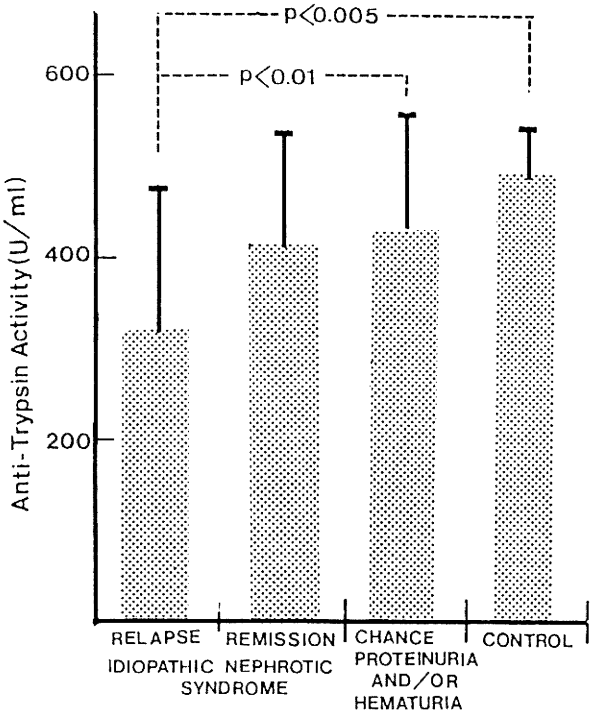

Fig 2. Plasma anti-trypsin activity.

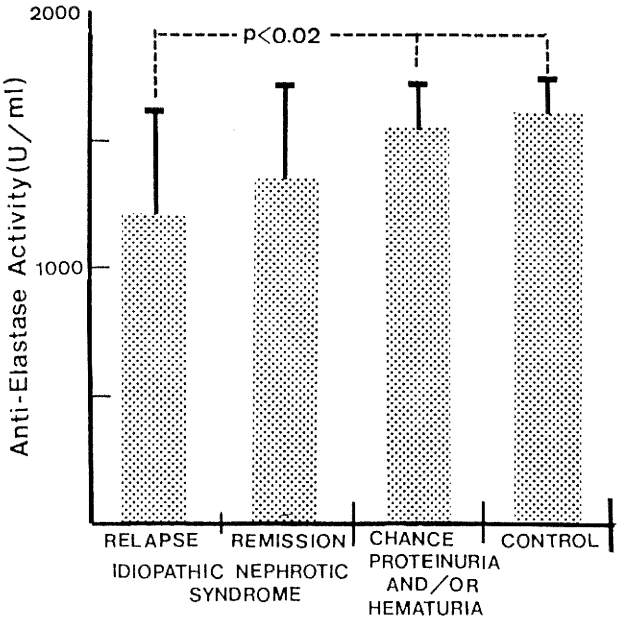

Fig 3. Plasma anti-elastase activity.

ml in INS during relapse, 1360.3±372.5U/ml in INS in remission, 1520.3±160.3 U/ml in CPH, and 1,604± 153.2 U/ml. The mean value in INS during relapse was significantly lower than that in CPH ($p<0.02$) and controls ($p<0.02$).

4. Relation between the protein concentrations of plasma α_1-PI and the proteinase inhibitory activities.

A correlation was found between the protein concentrations of plasma α_1-PI and the anti-trypsin activity in INS both during relapse ($r=0.71$, $p<0.01$) and in remission ($r=0.47$, $p<0.05$). There was no correlation between the values in CPH and in controls. A significant correlation was found between the protein concentrations of plasma α_1-PI and the anti-elastase activity in INS both during relapse ($r=0.51$, $p<0.05$) and in remission ($r=0.72$, $p<0.01$).

DISCUSSION

The possible relation between α_1-PI and renal disease was first suggested by Miller in a case report[10] of glomerulonephritis presented with congenital α_1-PI deficiency. Later, three cases of congenital α_1-PI deficiency were reported to have membranoproliferative glomerulonephritis with glomerular deposition of C3, IgM, and α_1-PI[11] or without glomerular de posits.[12] In addition, congenital nephrotic syndrome[13]

and rapidly progressive glomerulonephritis[14] were also reported to have developed in patients with congenital α_1-PI deficiency, suggesting a possible role for the inhibitor in preventing renal diseases.

In this study, plasma α_1-PI concentrations were significantly decreased in INS patients, not only during relapse but also in remission, indicating that INS patients have a lower inhibitory activity against some proteinases even in remission than normal subjects. However, since the degree of decrease in plasma α_1-PI activity was slight, the INS patients did not show any severe clinical manifestations as observed in congenital α_1-PI deficiency.[15,16]

Although the measured inhibitory effect of plasma α_1-PI on elastase was found to be decreased in INS both during relapse and in remission, the effect on trypsin was not decreased in remission. Several studies[6-8] have demonstrated decreased plasma α_1-PI concentrations in INS; however, the functional changes of the inhibitor have not been examined. The present study is the first to demonstrate both the decreased α_1-PI concentrations and the decreased inhibitory activity on elastase in remission of INS.

In aminonucleoside nephrotic rats, Davin et al.[1] has demonstrated that neutral proteinases might be involved in damage to the glomerular basement membrane (GBM) and in the subsequent development damage could be explained as follows: 1) Aminonucleoside can induce an *in-vivo* release of proteinases from leucocytes[12] 2) Neutral proteinases generated by cells derived from the glomerular mesangium are capable of degrading GBM glycoproteins *in-vitro*[13] 3) A metalloproteinase has also been recently detected in patients presenting with acute renal failure. A role for plasma proteinases in the degradation of GBM is therefore possible, despite the fact that their action is theoretically counteracted by the presence of strong plasma enzyme inhibitors[4] 4) Davin et al.[1] have demonstrated that urinary excretion of GBM components such as laminin and type IV collagen were increased in aminonucleoside nephrotic rats and that decreased GBM anionic sites were observed in these rats. Furthermore, a significant inhibition of neutral proteinase activity was observed with phenylethylsulfonylfluoride, soybean trypsin inhibitor, and aprotinin, but not with EDTA or cysteine, which suggested that the increased enzymatic activity mainly resulted from serine proteinases in these animals.[1]

The suggestion that the proteinases involved in the damage to the GBM might be one of the proteinases which could be inhibited by α_1-PI supports our hypothesis that the decreased plasma concentration and proteolytic activity of the plasma α_1-PI may be a predisposing factor to the development of INS. The molecular weight of α_1-PI is small (53.000), and α_1-PI is normally present in high concentrations in plasma.

In summary, we have demonstrated that the decreased plasma α_1-PI concentrations and decreased proteolytic activity are not only present during relapse but also in remission of INS. Under normal conditions, plasma α_1-PI may be acting as a defense against proteolytic injury of kidney tissue. In INS patients, however, lower plasma α_1-PI concentrations may result in proteolytic damage to kidney tissue, and in nephrotic syndrome as well.

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