Relationships between Urinary Levels of Ulinastatin and α_1 -Microglobulin and the Severity of Alzheimer-Type or Vascular Dementia

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Ulinastatin (urinary trypsin inhibitor) and α_1 -microglobulin levels in urine samples of 39 dementia patients (Alzheimer-type dementia, 21 cases; vascular dementia, 18 cases) and 14 normal subjects were measured. There was a positive correlation between the ulinastatin and α_1 -microglobulin levels. Lower scores on the dementia rating scale and activities of daily living were observed in patients with Alzheimer-type dementia but not with vascular dementia, where a lower α_1 -microglobulin level was registered. However, the ulinastatin level did not correlate with the scores in patients with either Alzheimer-type or vascular dementia. These results indicate that the urinary α_1 -microglobulin level provides a useful biochemical index of the severity of Alzheimer-type dementia.

Key words: urinary trypsin inhibitor, ulinastatin, α_1 -microglobulin, Alzheimer-type dementia, vascular dementia

Introduction

The amino acid sequence of ulinastatin (UT), a trypsin inhibitor found in human urine, is highly homologous for the protease inhibitory domain of amyloid β -protein precursor¹⁻³⁾. The importance of this domain in β -amyloid deposition in the brain has been demonstrated⁴⁾.

Moreover, a UT-like immunoreactive substance has been discovered in brain tissues of subjects with senile Alzheimer-type dementia⁵⁾. Similar to UT, α_1 -microglobulin (α_1 -M) in various body fluids⁶⁾ is processed from the same precursor protein^{7,8)}, and variation of the α_1 -M level in the cerebrospinal fluid has been noted in certain neurological disorders⁹⁾. In the present experiment, we investigated the relationships between the urinary levels of UT and α_1 -M and the severity of Alzheimer-type dementia (ATD) or vascular dementia (VD). Our results indicate

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Tab. 1 Sex, Age and Levels of Creatinine and Protein in Urine of Subjects

	Nondemented controls (NC)	Alzheimer-type dementia (ATD)	Vascular dementia (VD)
Number of subjects	14	21	18
Sex Male	8	5	8
Female	6	16	10
Age (years)	72 $\pm$ 2	75 $\pm$ 2	76 $\pm$ 3
Creatinine (mg/ml)	0.853 $\pm$ 0.109	0.863 $\pm$ 0.056	0.853 $\pm$ 0.091
Protein (mg/ml)	0.118 $\pm$ 0.026	0.100 $\pm$ 0.013	0.110 $\pm$ 0.016

Values in age and urinary levels of creatinine and protein are represented as mean $\pm$ SEM.

that the urinary level of α_1 -M is closely correlated to the severity of ATD.

Methods

Subjects

Twenty-one ATD and 18 VD patients (total: 39 subjects) were diagnosed according to an established criteria¹⁰⁾ and VD classification¹¹⁾. Fourteen nondemented controls (NC) without any history or symptoms of psychiatric, neurological or malignant diseases were included as reference cases. The patient profiles and biochemical data are shown in Table 1.

Assay of urine sample

Urine was spontaneously collected from the subjects, followed by rapid centrifugation at 1,000 $\times$ g for 10 min to remove debris and amorphous salts. Samples were then stored at -20°C until assay. UT levels were measured by the sandwich enzyme immunoassay with polyclonal

rabbit anti-UT-IgG¹²⁾. Standard UT was donated by the Mochida Pharmaceutical Co., Japan. Urinary α_1 -M and creatinine contents were measured by the latex agglutination photometric immunoassay¹³⁾ using an automated instrument (LX-300, Eiken Co., Tokyo) and a photometric assay based on the Jaffe reaction¹⁴⁾ (Wako creatinine test-kit, Wako Pure Chemical Ind., Osaka), respectively. Standard α_1 -M and anti α_1 -M rabbit IgG-coated latex were obtained from Eiken Co. Protein contents in the urine were measured by the method of Bradford¹⁵⁾ with bovine serum albumin as the standard. In correlating the urinary level of UT or α_1 -M with scores from either the dementia rating scale or activities of daily living, contents of the former endogenous substances were expressed as $\mu\text{g}/\text{mg}$ creatinine in urine to correct for the dilution rate of urine samples.

Severity of dementia

The severity of dementia was evaluated using Hasegawa's dementia rating scale¹⁶⁾ and scale of activities of daily living as categorized by the Japanese Association of Psychiatric Hospitals¹¹⁾. According to Hasegawa's dementia rating scale, the maximum score was 32.5. Scores of normal, subnormal, pre-dementia and dementia were graded as >31 , 30.5-22, 21.5-10.5 and <10 ,

Abbreviations:

UT=ulinastatin
 α_1 -M= α_1 -microglobulin
 ATD=Alzheimer-type dementia
 VD=vascular dementia
 NC=nondemented controls
 DRS=dementia rating scale
 ADL=activities of daily living
 Cr=creatinine

Fig. 1 Correlation between the concentration of α_1 -microglobulin and that of ulinastatin in urine of nondemented controls (NC) and patients with either Alzheimer-type dementia (ATD) or vascular dementia (VD). R and n in parentheses represent the correlation coefficient and number of subjects, respectively.

Fig. 2

Correlation between scores from the dementia rating scale and activities of daily living of patients with Alzheimer-type dementia (ATD) or vascular dementia (VD). R and n in parentheses represent the correlation coefficient and number of subjects, respectively. Maximum scores for the dementia rating scale and activities of daily living were 32.5 and 65, respectively.

respectively. In the scale of activities of daily living, the ability to perform the following 13 items was graded from 1 to 5: feeding, toileting (defecation and urination), transfer, ambulation, sphere of walking, bathing, dressing, cleaning (and tidiness), hearing, visual acuity, emotional expression and verbal comprehension. For cases who were incapable and/or totally dependent on assistants, the score was graded as 1, whereas normal and/or fully independent patients were

graded as 5. The maximum and minimum scores for the former and latter were 65 and 13, respectively.

Statistical analysis

Statistical analyses were performed by using the Student's t-test.

Results

There was no significant difference in age

Fig. 3

Correlation between the urinary level of either α_1 -microglobulin (α_1 -M) or ulinastatin (UT) and the score of dementia rating scale (DRS) or activities of daily living (ADL) of patients with Alzheimer-type dementia. Levels of α_1 -M and UT were expressed as $\mu\text{g}/\text{mg}$ creatinine in urine. R and n in parentheses represent the correlation coefficient and number of subjects, respectively.

Fig. 4

Correlation between the urinary level of either α_1 -microglobulin (α_1 -M) or ulinastatin (UT) and the score of the dementia rating scale (DRS) or activities of daily living (ADL) of patients with vascular dementia. Levels of α_1 -M and UT were expressed as $\mu\text{g}/\text{mg}$ creatinine in urine. R and n in parentheses represent the correlation coefficient and number of subjects, respectively.

between any two cases of the NC and dementia groups (Table 1). In ATD patients, females outnumbered males, whereas the number of males and females was almost equal in VD patients. The urinary levels of creatinine and protein in dementia patients were similar to those of NC.

Figure 1 reveals the existence of a positive correlation between the UT and α_1 -M concentrations in urine. The correlation coefficients in NC, ATD and VD groups were 0.77 ($p < 0.01$), 0.51 ($p < 0.05$) and 0.69 ($p < 0.01$), respectively.

Dementia patients with lower scores on the dementia rating scale indicated a similar tendency in the activities of daily living (Figure 2). There was a positive correlation between these scores both in ATD and VD. The correlation coefficients in ATD and VD were 0.48 ($p < 0.05$) and 0.58 ($p < 0.05$), respectively.

In ATD patients, the urinary α_1 -M level was significantly and positively correlated with scores from both the dementia rating scale ($p < 0.05$) and activities of daily living ($p < 0.05$), whereas no correlation was noted between the UT level and either of these scores (Figure 3). However, neither α_1 -M nor UT levels in urine indicated any correlation with these scores in the VD patients (Figure 4).

Discussion

Both human α_1 -M and UT are derived from a common precursor protein by intracellular proteolytic processing⁷⁾, and a gene which encodes the precursor protein has been reported^{17,18)}. This precursor protein is synthesized in the liver^{8,19)}. Some UT derived from such precursor protein serves as a component of the matured plasma inter- α -trypsin inhibitor⁸⁾ or plasma pre- α -trypsin inhibitor²⁰⁾ whereas the remaining UT exists as a serum trypsin inhibitor²¹⁾. The protein, α_1 -M displays immunosuppressive activity²²⁾, and exists in both free and IgA-complexed forms

in plasma¹³⁾. In the present study, the α_1 -M level was found to positively correlate with UT level in the urine (Figure 1), indicating that both of these substances were derived from a common precursor protein. Assuming the respective molecular weights of α_1 -M and UT are 31 kDa¹³⁾ and 40 kDa¹²⁾, the regression equation is $Y = 0.78X$ (where Y and X represent urinary levels of α_1 -M and UT, respectively) if these two substances are excreted at a similar molar ratio. However, in fact, the regression equations for NC, ATD and VD groups were $Y = 0.85X - 2.91$, $Y = 0.91X - 2.43$ and $Y = 1.56X - 6.96$, respectively. These findings suggest that UT and α_1 -M are not excreted at the same molar ratio. The difference in the excretion ratio may have been caused by a variation in the quantity of their free forms in plasma and/or reabsorption in the urinary tract.

As 31 kDa α_1 -M is filtered out by the glomeruli and is extensively absorbed at the proximal tubular cells, the urinary α_1 -M level is used in the diagnosis of renal tubular dysfunction¹³⁾. In addition, α_1 -M levels also reflect dynamic changes in syntheses by the liver and complex formations in serum¹³⁾. In the present study, creatinine clearance was not obtained due to the difficulty to measure in dementia patients, however, the creatinine and protein levels in the urine samples of dementia patients were similar to those in NC (Table 1). In addition, in the protein profile determined with SDS-electrophoresis of urine samples of NC and dementia patients, certain similar protein bands were detected in both urine samples of the former and latter (data not shown). Thus, renal dysfunction in dementia patients is not likely a dominant factor influencing the correlation between the urinary level of α_1 -M (or UT) and dementia severity. When both α_1 -M and UT levels in the urine were expressed as $\mu\text{g}/\text{mg}$ creatinine, considerable

variations in these levels were observed among the patients. The range of levels in dementia patients was more extensive than that in NC. The percentage of patients whose urinary levels of α_1 -M and UT were within the range of NC were 43 and 86% of ATD subjects, and 56 and 67% of VD patients, respectively. Thus, the urinary level of α_1 -M or UT per se would not provide useful information for the dementia diagnosis. However, in the present study, the severity of ATD based on the scores of the dementia rating scale and activities of daily living, was correlated with the α_1 -M but not with the UT level. Furthermore, neither of these levels was correlated with the disease severity in VD patients. Although, the exact mechanism(s) of these findings warrants further study, it has been reported that a principal feature of the ATD process is expressed subclinically in tissues other than the brain^{23,24}. In the correlation between urinary levels of α_1 -M in ATD patients and scores on the dementia rating scale (or activities of daily living), the significant ($p < 0.05$) coefficient value was 0.54 (or 0.48 for activities of daily living; $p < 0.05$). The comparatively low value of this coefficient value may have been attributed to the complexity of dementia severity, which is influenced by many factors. In conclusion, the present findings implicate that the urinary α_1 -M level may serve as a biochemical index for monitoring ATD development.

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