Nonexistence of a positive correlation between urinary levels of α1-microglobulin and ulinastatin in patients with Parkinson's disease

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Running title: α1M and UT in Parkinson's disease

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Abstract

Urinary levels of α1-microglobulin (α1M) and ulinastatin (UT) and α1M/UT ratio did not differ significantly (i) between age-matched control and Parkinson's disease groups and (ii) among subdivided groups based on Yahr's stages in Parkinson's disease. Further, these indexes did not correlate well with Yahr's stages. Although α1M and UT levels did not correlate in patients with Parkinson's disease, a positive correlation was observed in the control group. The nonexistence of a positive correlation between α1M and UT levels distinguishes Parkinson's disease from other neuropsychiatric diseases such as dementia (Alzheimer-type and vascular dementia), schizophrenia and mood disorder.

INTRODUCTION

Alpha-1-microglobulin (α1M) and ulinastatin* (UT) are found in human urine. Although these two substances are derived from a common precursor protein in the liver,[1] they are structurally unrelated glycoproteins. While α1M displays immunosuppresive activities[1] and UT elicits trypsin inhibitory activities[2], their physiologically relevant functions are still obscure. We have previously found that the relationship between urinary α1M and UT levels of dementia group differed from that of the age-matched non-demented control, and in the relation of α1M/UT ratios to the severity of dementia and duration of the disease, different patterns were noted between the Alzheimer-type senile dementia and vascular dementia.[3] In the present study, the relationship between urinary α1M and UT levels in patients with Parkinson's disease were investigated.

MATERIALS AND METHODS

Sampling and storage

Urine was spontaneously collected from healthy subjects without

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* The generic name of urinary trypsin inhibitor has been recently changed from urinastatin to ulinastatin, as the substance is found in not only urine but also serum.

any history or symptoms of psychiatric and neurological diseases and in- or out-patients suffering from Parkinson's disease in Shimane Medical University. Samples were centrifuged at 1,000 x g for 10 min at 4°C prior to storage at -50°C until assay. The severity of Parkinson's disease was evaluated according to Yahr's stages,[4] and none of the subjects indicated hepatic and renal dysfunctions or other malignant diseases. Informed consent was obtained from all subjects after the purpose of the present study was explained to them.

Determination of α1M, UT and creatinine contents

Urinary α1M and UT levels were measured (ELISA method) by interacting galactosidase-labelled goat antirabbit IgG (Biotrin International, Doublin) with rabbit anti-α1M IgG (Dako Co., Copenhagen) and rabbit anti-UT IgG,[5] respectively. Standard α1M (Dako Co., Copenhagen) and UT (a kind gift from Mochida Pharmaceutical Co., Tokyo) were used as references. Creatinine contents in the urine were measured with a Wako Creatinine Kit (Wako Chemicals, Osaka). Urinary α1M and UT contents were expressed as μg/mg creatinine to correct for the dilution rate of urine samples.

Statistical analysis

Significant differences of α1M and UT contents and α1M/UT ratios between healthy subjects and patients with Parkinson's disease and among the three subdivided groups based on Yahr's stages were verified with the unpaired t-test and factorial ANOVA followed by Scheffe's post hoc test, respectively. Linear regression analyses and Spearman's rank correlation between the stages in patients with Parkinson's disease and respective α1M and UT levels and α1M/UT ratios were evaluated by using the Stat View IV (Abacus Concept, Inc., CA). P values of less than 0.05 were considered significant.

RESULTS

The α1M and UT levels and α1M/UT ratio between the control and Parkinson's disease groups and among the three subdivided groups (based on Yahr's stages) did not differ significantly (Table 1). With regard to subjects (composed exclusively of female patients) in both the control and Parkinson's disease groups, the α1M, UT levels and α1M/UT ratio did not display any statistical significances. In the patients with Parkinson's disease, significant correlations between the stages and respective α1M and UT levels and α1M/UT ratios were not observed when the Spearman's rank correlation was employed.

The existence of a positive correlation between the UT and α1M levels in urine was significantly observed in the control but not Parkinson's disease group (Figure 1). Female subjects both in the control and Parkinson's disease groups showed a pattern similar to that depicted in Figure 1. The correlation coefficients between the UT and α1M contents in the control and Parkinson's disease groups were 0.939 (p value in the regression, $P<0.002$; $n=7$) and 0.195 (p value in the regression, $P=0.505$; $n=14$), respectively.

DISCUSSION

Significant differences in the urinary α1M and UT levels and α1M/UT ratios were not detected between the control and Parkinson's disease groups. A similar tendency was indicated among the subdivided groups of patients with Parkinson's disease, though the cases of each stage were small in number. Furthermore, there was no significant correlation between the severity of Parkinson's disease and the respective levels of α1M and UT and α1M/UT ratios. These findings suggest that urinary levels of α1M and UT and the α1M/UT ratio per se cannot serve as useful indexes for the diagnosis and monitoring of the development of Parkinson's disease.

Contrary to controls, demented patients (both Alzheimer-type dementia and vascular dementia),[3] patients with schizophrenia and mood disorder (both bipolar affective disorder and major depression) (unpublished data), a positive correlation between urinary levels of α1M and UT was not observed in the patients with Parkinson's disease. Regarding nonexistence of the positive correlation, renal factors might not be a factor, since positive correlation is observed even in patients with urological diseases,[6] and none of the present subjects indicated any renal dysfunctions. In our investigation with C57 BL/6J mice, an animal species reportedly possesses the gene similar to that of humans for synthesizing the precursor protein of α1M and UT,[7] L-dopa treatment (50 mg/kg, i.v.; 25 mg/kg/day, s.c. for 7 consecutive days) did not affect the positive correlation in non-treated mice, whereas repetitive MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine produces pathological changes similar to human idiopathic Parkinson's disease in animals[8]) administrations (20 mg/kg, i.p., 4 injections every 12 hours) resulted in nullifying the positive correlation (unpublished data). These results suggest that the nonexistence of the correlation is closely associated with the neuropathological changes in Parkinson's disease, but not with L-DOPA treatment, though all the patients with Parkinson's disease were medicated

with L-DOPA. Although the exact mechanism warrants further studies, our present findings revealed an interesting feature between urinary levels of α1M and UT in Parkinson's disease.

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Table 1. Number, sex, age, levels of α1-microglobulin (α1M) and ulinastatin (UT), and α1M/UT ratios in urine of normal and patients with Parkinson's disease

	Normal control	Parkinson's disease			
		stage 2	stage 3	stage 4	Total
Number of subjects	11	3	7	5	15
Sex (M/F)	4 / 7	1 / 2	0 / 7	0 / 5	1 / 14
Age (years)	63 ± 2 (64 ± 1)	50 ± 10	63 ± 3	62 ± 7	60 ± 3 (61 ± 3)
α1M (μg/mg Cr.)	11.01 ± 2.15 (12.41 ± 3.09)	5.01 ± 2.38	8.49 ± 2.28	5.66 ± 2.20	6.85 ± 1.28 (6.81 ± 1.38)
UT (μg/mg Cr.)	8.12 ± 1.57 (7.66 ± 1.91)	5.35 ± 3.94	6.77 ± 1.12	8.25 ± 2.34	6.98 ± 1.04 (6.66 ± 1.06)
Ratio (α1M/UT)	1.51 ± 0.21 (1.71 ± 0.23)	1.53 ± 0.62	1.47 ± 0.49	0.76 ± 0.25	1.25 ± 0.26 (1.29 ± 0.27)

Patients with Parkinson's disease were subdivided based on Yahr's stages. Values in age and urinary levels of α1M and UT and α1M/UT ratio are represented as mean ± S.E.M.. Values in parentheses are those of female cases. M, male; F, female; Cr., creatinine in urine.

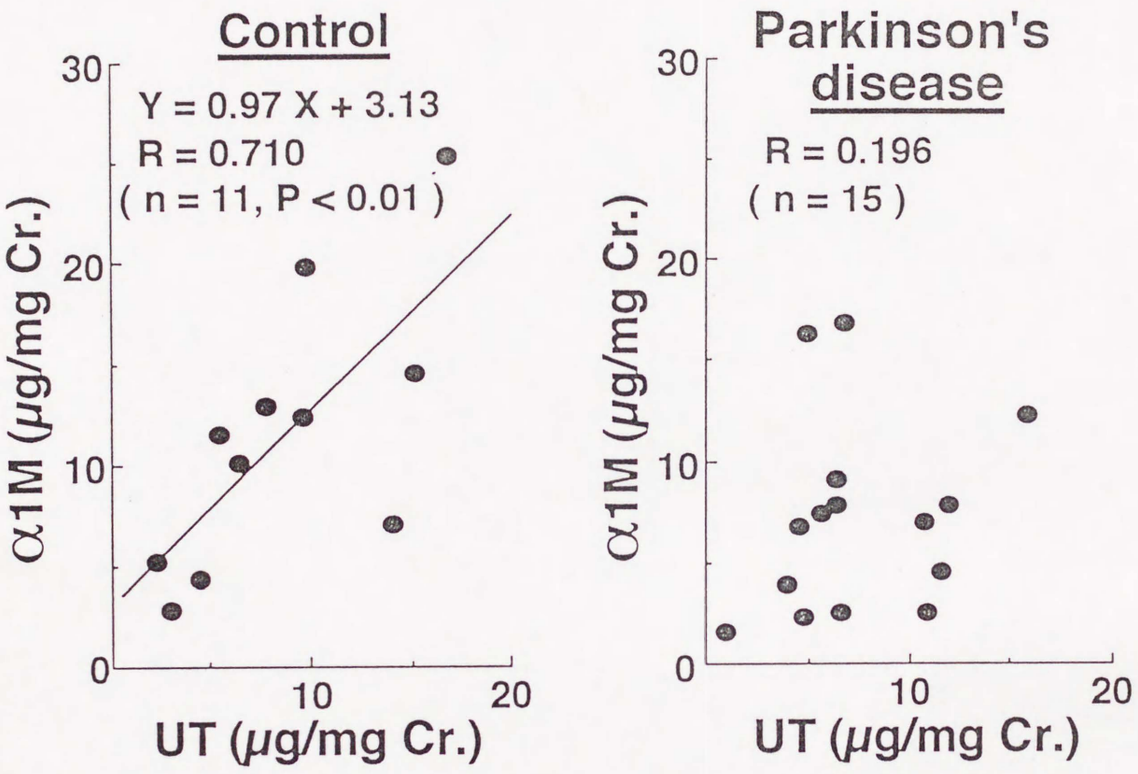

Figure 1. Correlation between urinary levels of UT and α1M in age-matched normal controls and patients with Parkinson's disease. R and n in parentheses represent the correlation coefficient and number of subjects, respectively. P value shows the significance of the regression.