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Plasma serotonin is a predictor for deterioration of urinary albumin excretion in men with type 2 diabetes mellitus

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Abstract

We performed an observational study to investigate if plasma 5-hydroxyindole-3-acetic acid (5-HIAA), a derivative end product of serotonin (5-hydroxytryptamine), concentration could be a predictor for deterioration of urinary albumin excretion. The relationship between baseline plasma 5-HIAA concentration and changes in urinary albumin excretion for 24 months was investigated in 162 male patients with type 2 diabetes mellitus. Patients were divided into tertiles according to plasma 5-HIAA concentration. Greater changes in urinary albumin excretion were seen in patients with high plasma 5-HIAA concentration (112.8 ± 36.2 mg/g creatinine) than in patients with low plasma 5-HIAA concentration (7.6 ± 8.0 mg/g creatinine, $P = .0011$) or in patients with intermediate plasma 5-HIAA concentration (25.6 ± 15.0 mg/g creatinine, $P = .0070$) after adjustment for baseline values of urinary albumin excretion. A positive correlation was observed between log (plasma 5-HIAA concentration) and changes in urinary albumin excretion ($r = 0.314$, $P < .0001$). Multiple regression analysis demonstrated that log (plasma 5-HIAA concentration) ($\beta = .284$, $P = .0013$) was an independent determinant of changes in urinary albumin excretion. In conclusion, plasma 5-HIAA concentration was positively correlated with changes in urinary albumin excretion, which may indicate causality in diabetic nephropathy in male patients with type 2 diabetes mellitus and high plasma 5-HIAA concentration.

1. Introduction

Cardiovascular disease (CVD) is the leading cause of mortality and morbidity in patients with type 2 diabetes mellitus; and several risk factors, including smoking, hypertension, and dyslipidemia, have been shown to accelerate the progression of CVD [1-3]. Male sex is an independent risk factor for CVD [4]. Moreover, elevated urinary albumin excretion, which is a useful marker of diabetic nephropathy, has been reported to be associated with increased risk of cardiovascular mortality [5]. Serotonin (5-hydroxytryptamine [5-HT]) mediates vasoconstriction and induces the activation of platelets, which may promote atherosclerosis. Plasma 5-HT concentration has been reported to be high in diabetic patients [6,7], which may be one of the underlying mechanisms of diabetic complications. 5-Hydroxytryptamine$_{2A}$ receptor has been identified in glomerular mesangial cells [8], which suggests the involvement of 5-HT in the development of diabetic nephropathy through proliferation and matrix synthesis in mesangial lesions. We therefore performed an observational study to investigate the relationship between plasma 5-hydroxyindole-3-acetic acid (5-HIAA), a derivative end product of 5-HT, concentration and changes in urinary albumin excretion in male patients with type 2 diabetes mellitus.

2. Subjects and methods

2.1. Patients

The relationship between plasma 5-HIAA concentration and changes in urinary albumin excretion was investigated in 162 male patients with type 2 diabetes mellitus recruited from the outpatient clinic at the Kyoto Prefectural University of Medicine. The relationships between changes in urinary albumin excretion and major cardiovascular risk factors,

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including blood pressure, glycemic control (hemoglobin A_{1c} [HbA_{1c}]), and body mass index (BMI), were also evaluated. Baseline plasma 5-HIAA concentration and urinary albumin excretion were measured in 2006. After 24 months, urinary albumin excretion was measured; and any changes in urinary albumin excretion were calculated. Patients were divided into 3 groups according to plasma 5-HIAA concentrations.

2.2. Clinical and biochemical assessment

Type 2 diabetes mellitus was diagnosed according to the "Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus" [9]. Patients were excluded if they took any medications that might affect plasma 5-HIAA concentration (eg, 5-HT receptor blockers) or began to take angiotensin-converting enzyme inhibitor and/or angiotensin II receptor blocker during the study. Patients with advanced renal dysfunction (serum creatinine more than 2.0 mg/dL) were also excluded. Approval for the study was obtained from the local Research Ethics Committee, and informed consent was obtained from all participants.

Blood and urine samples were obtained in the morning. Plasma 5-HIAA concentrations (reference range, 1.8-6.1 ng/mL) were measured by high-performance liquid chromatography. The intraassay coefficients of variation were 2.1%, 2.0%, and 0.9% for plasma 5-HIAA concentrations of 25.27, 41.30, and 95.09 ng/mL, respectively. The interassay coefficients of variations were 3.9%, 3.3%, and 2.4% for plasma 5-HIAA concentrations of 7.45, 20.55, and 60.83 ng/mL, respectively. Urinary albumin excretion was measured with an immunoturbidimetric assay. Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride concentrations were assessed using standard enzymatic methods. Hemoglobin A_{1c} was assayed by high-performance liquid chromatography.

2.3. Statistical analysis

Means and frequencies of potential confounding variables were calculated. Unpaired Student t tests or analyses of variance were conducted to assess statistical significance of differences between groups using StatView software (version 5.0; SAS Institute, Cary, NC). To minimize the potential influence of differences in baseline values of urinary albumin excretion when we assessed statistical significance of changes in urinary albumin excretion, analysis of covariance was used. Because plasma 5-HIAA concentration showed skewed distributions, logarithmic (log) transformation was carried out before performing correlation and regression analysis. The relationship between log (plasma 5-HIAA concentration) and changes in urinary albumin excretion was examined by Pearson correlation analyses. Multiple regression analysis was performed to assess the combined influence of variables on changes in urinary albumin excretion. To examine the effects of various factors on changes in urinary albumin excretion, the following factors were considered as independent variables: log (plasma 5-HIAA concentration); age; duration of diabetes; HbA_{1c}; BMI; systolic blood pressure; diastolic blood pressure; serum total cholesterol, triglyceride, and HDL cholesterol concentration; and smoking status. A P value less than .05 was considered statistically significant.

3. Results

Clinical characteristics of the 162 male patients with type 2 diabetes mellitus enrolled in this study are shown in Table 1. Greater changes in urinary albumin excretion were seen in patients with high plasma 5-HIAA concentration (112.8 ± 36.2 mg/g creatinine) than in patients with low plasma 5-HIAA concentration (7.6 ± 8.0 mg/g creatinine, $P = .0011$) or in patients with intermediate plasma 5-HIAA concentration (25.6 ± 15.0 mg/g creatinine, $P = .0070$) after adjustment for baseline values of urinary albumin excretion (Fig. 1). A positive correlation was observed between log (plasma 5-HIAA concentration) and changes in urinary albumin excretion ($r = 0.314$, $P < .0001$) (Fig. 2). Multiple regression analysis demonstrated that log (plasma 5-HIAA concentration) ($\beta = 0.284$, $P = .0013$) was an independent determinant of changes in urinary albumin excretion ($R^2 = 0.369$).

4. Discussion

The present data suggest that plasma 5-HIAA, a derivative end product of 5-HT, concentration is a predictor of deterioration of urinary albumin excretion in male patients with type 2 diabetes mellitus. Greater changes in urinary albumin excretion were seen in patients with high plasma 5-HIAA concentration than in patients with low plasma 5-HIAA concentration, and a significant positive correlation was observed between log (plasma 5-HIAA concentration) and changes in urinary albumin. In addition, log (plasma 5-HIAA concentration) was an independent factor to determine changes in urinary albumin excretion, even for a 24-month period, in male patients with type 2 diabetes mellitus according to the present multiple regression analysis.

Patients who had more initial urinary albumin excretion might show wider range of changes in urinary albumin excretion. However, initial urinary albumin excretion was not significantly different among 3 groups according to plasma 5-HIAA concentrations; and initial urinary albumin excretion was not correlated significantly with log (plasma 5-HIAA concentration) in the present study. Moreover, after adjustment for baseline values of urinary albumin excretion, greater changes in urinary albumin excretion were seen in patients with high plasma 5-HIAA concentration than in patients with low plasma 5-HIAA concentration or in patients with intermediate plasma 5-HIAA concentration; and a positive correlation was observed between log

Table 1
Clinical characteristics of patients with diabetes

Characteristic	All patients	Plasma 5-HIAA		
		Low	Intermediate	High
n	162	54	54	54
Age (y)	63.9 ± 11.1	58.6 ± 12.0	65.6 ± 10.1†	67.5 ± 9.0†
Age at onset (y)	50.3 ± 12.5	48.2 ± 13.1	50.8 ± 12.1	52.1 ± 12.2
Duration of diabetes (y)	13.4 ± 11.5	10.4 ± 10.3	15.2 ± 12.0*	14.7 ± 12.0
BMI (kg/m^2)	23.0 ± 3.2	23.7 ± 3.7	23.1 ± 2.7	22.4 ± 3.0*
HbA_{1c} (%)	7.1 ± 1.1	7.0 ± 1.1	7.2 ± 1.1	7.0 ± 1.1
Systolic blood pressure (mm Hg)	132 ± 15	132 ± 14	132 ± 14	130 ± 17
Diastolic blood pressure (mm Hg)	76 ± 10	80 ± 9	76 ± 8	73 ± 12†
Total cholesterol (mg/dL)	192 ± 31	197 ± 31	197 ± 27	182 ± 33*,‡
Triglyceride (mg/dL)	125 ± 78	127 ± 89	114 ± 55	135 ± 86
HDL cholesterol (mg/dL)	52 ± 15	52 ± 15	54 ± 15	49 ± 13‡
Smoking (none/past/current)	33/91/38	8/27/19	15/30/9	10/34/10
Current treatment (diet/OHA/insulin)	22/97/43	12/30/12	2/37/15	8/30/16
5-HIAA (ng/mL)	6.9 ± 5.4	3.5 ± 0.8	5.7 ± 0.7†	11.6 ± 7.3†,§
Range of 5-HIAA (ng/mL)	1.4-52.8	1.4-4.5	4.6-7.1	7.2-52.8

Data are mean ± SD or number of patients. OHA indicates oral hypoglycemic agent.
* $P < .05$ vs low 5-HIAA.
† $P < .01$ vs low 5-HIAA.
‡ $P < .05$ vs intermediate 5-HIAA.
§ $P < .01$ vs intermediate 5-HIAA.

(plasma 5-HIAA concentration) and changes in urinary albumin excretion.

Albuminuria may be a convenient marker of diffuse endothelial dysfunction [10] and may serve as a marker of CVD [11,12] as well as nephropathy, being likely to reflect both macrovascular and microvascular disease. 5-Hydroxytryptamine induces the contraction, migration, and proliferation of vascular smooth muscle cell via the 5-HT_{2A} receptor followed by various intracellular signal transduction mechanisms [13-15]. Moreover, Kasho et al [16] demonstrated that 5-HT increased the production of type 4 collagen by cultured human mesangial cells through 5-HT_{2A} receptor, which was mediated by activation of protein kinase C and subsequent increase in transforming growth factor–β activity. Currently, sarpogrelate hydrochloride, a potent 5-HT_{2A} receptor antagonist that inhibits 5-HT–induced vasoconstriction and platelet aggregation [17], is used clinically as an antiplatelet drug for the prevention of thrombosis in atherosclerotic disease. Takahashi et al [18] reported that sarpogrelate hydrochloride reduced the degree of urinary albumin excretion, indicating the potential usefulness of this agent for the protection of development and progression of diabetic nephropathy.

Age in the high group of plasma 5-HIAA concentration was significantly higher than that of the low group. However,

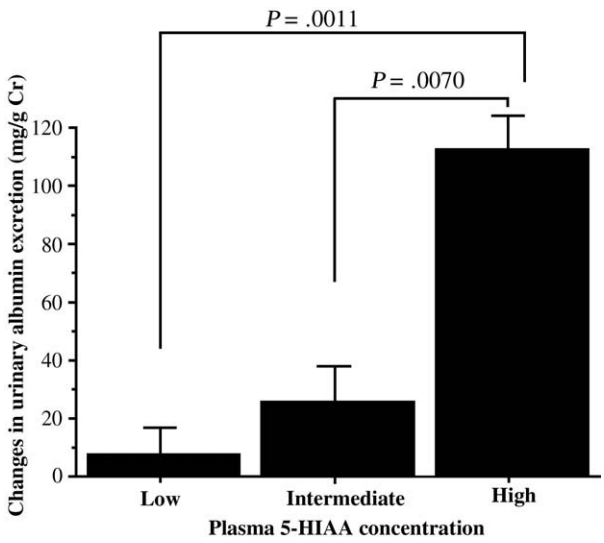

Fig. 1. Changes in urinary albumin excretion in tertiles of plasma 5-HIAA concentration in male patients with type 2 diabetes mellitus. Data are mean ± SE.

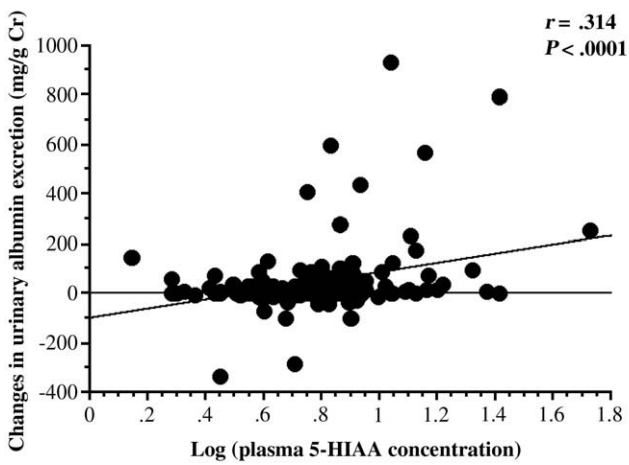

Fig. 2. Correlation between log (plasma 5-HIAA concentration) and changes in urinary albumin excretion in male patients with type 2 diabetes mellitus.

even after adjustment for age, greater changes in urinary albumin excretion were seen in patients with high plasma 5-HIAA concentration than in patients with low plasma 5-HIAA concentration; and a positive correlation was observed between log (plasma 5-HIAA concentration) and changes in urinary albumin excretion.

Limitations of our study include a lack of detailed information concerning plasma 5-HIAA concentration, including whether there is circadian variation or not and the correlation between plasma 5-HIAA concentration and plasma 5-HT concentration. We obtained blood and urine samples in the morning, which may reduce the effect of circadian variation, if any. Nityanand et al [19] reported concomitant increase in plasma 5-HIAA concentration and plasma 5-HT concentration in patients with hypertension. Certainly, deteriorated kidney function would affect plasma 5-HIAA concentration. However, we excluded patients with advanced renal dysfunction (serum creatinine >2.0 mg/dL) in this study. Moreover, the main findings in this study did not change even in patients with normal serum creatinine level (data not shown). Despite a lack of those detailed information concerning plasma 5-HIAA concentration, it is much easier and clearer in medical practice to measure plasma 5-HIAA, a derivative end product of 5-HT, instead of 5-HT, which is difficult to measure. In addition, we selected only men as study subjects because male sex is an independent risk factor for CVD [4]; and elevated urinary albumin excretion, which is a useful marker of diabetic nephropathy, has been reported to be associated with increased risk of cardiovascular mortality [5]. We should investigate if this finding is also applicable to female patients with type 2 diabetes mellitus. However, to our knowledge, this is the first study to investigate the relationship between plasma 5-HIAA concentration and changes in urinary albumin excretion. Plasma 5-HIAA concentration may be a predictor of changes in urinary albumin excretion in male patients with type 2 diabetes mellitus. Longer-term prospective trials and interventional studies with larger samples are needed to better assess the relationship between plasma 5-HIAA concentration and changes in urinary albumin excretion.

In conclusion, plasma 5-HIAA concentration was positively correlated with changes in urinary albumin excretion, which may indicate causality in diabetic nephropathy in male patients with type 2 diabetes mellitus and high plasma 5-HIAA concentration.

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