Determination of D-Amino Acids in Serum from Patients with Renal Dysfunction

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D-Ala and D-Ser were detected in the sera of both normal subjects and patients with renal dysfunction, and their concentrations were higher in the patients than in the normal subjects. A positive correlation between the concentration of D-Ala or D-Ser and that of creatinine (r = 0.733, p < 0.001 or r = 0.634, p < 0.001) or blood urea nitrogen (BUN) (r = 0.449, p < 0.05 or r = 0.629, p < 0.001) was observed in sera from 20 patients with renal dysfunction. The fraction (%D) of D-Ala in the total Ala in serum ([D/(D + L)] x 100) correlated well with the concentration of creatinine (r = 0.811, p < 0.001), suggesting that it is a candidate as a marker for renal proximal tubular dysfunction. The correlations of %D of Ser with creatinine and BUN levels were 0.796 (p < 0.001) and 0.919 (p < 0.001), respectively, indicating that %D of Ser may reflect protein turnover or catabolism in certain tissues as well as renal proximal tubular dysfunction.

Key words D-amino acid; HPLC; 4-fluoro-7-nitro-2,1,3-benzoisadiazole; renal proximal tubular dysfunction; creatinine; blood urea nitrogen (BUN)

Although L-amino acids have long been considered the sole amino acid constituents in mammalian bodies, D-amino acids have also been found recently in mammals.1–8) In the previous paper,9) we detected D-Ala in the serum of normal subjects by high-performance liquid chromatography (HPLC) with a Pirkle type chiral stationary phase. Nagata10) and Bruckner et al.11) have reported that serum levels of D-amino acids are related to degrees of renal dysfunction. In this paper, we examined the levels of D-amino acids in serum from patients and investigated their correlation with the concentration of serum creatinine or of blood urea nitrogen (BUN), both of which are clinical markers for renal dysfunction.

MATERIALS AND METHODS

Chemicals D- and L-amino acids were purchased from Kyowa Hakko Kogyo (Tokyo, Japan). Citric acid monohydrate was from Kanto Kagaku Co., Ltd. (Tokyo, Japan). Sumichiral OA-4600 (S,S) and OA-4700 (S,R) (250 x 4.6 mm i.d. 5 μm) were donated by Sumika Analytical Center Co., Ltd. (Osaka, Japan). 4-Fluoro-7-nitro-2, 1, 3-benzoisadiazole (NBD-F)12) was purchased from Tokyo Kasei Co., Ltd. (Tokyo, Japan). Methanol for the mobile phase was of HPLC grade (Wako Pure Chemicals Co., Ltd., Osaka, Japan).

Sample Preparation of Serum The sera of patients with renal dysfunction were obtained from the Branch Hospital, University of Tokyo. The serum was treated according to our previous method.9) Briefly, 10 μl of the serum and 90 μl of methanol were mixed vigorously for deproteinization. After centrifugation at 1000 x g for 5 min, 10 μl of the supernatant was added to 10 μl of 0.2 M borate buffer (pH 8.0) containing 4 mM disodium ethylenediaminetetraacetate (EDTA-2Na). Then, 30 μl of 50 mM NBD-F in acetonitrile was added and heated at 60℃ for 5 min, and 250 μl of 1% acetic acid in methanol was added to the solution. The resultant mixture was passed through a 0.5 μm membrane filter (Nihon Millipore, Japan) and 10 μl of the filtrate was injected into the HPLC system.

The coefficient of variation (c.v.%) between intra-day assay was 4.5–4.9% at 2.5 μM D-Ala in serum (n = 4), and the detection limit was approximately 10 fmol on a column (signal to noise ratio 3).

HPLC Equipment The HPLC system included an L-6200 intelligent pump, F-1050 fluorometric detector and D-2000 integrator (Hitachi, Tokyo, Japan). The column temperature was ambient. An analytical column of OA-4600 (S,S) or OA-4700 (S,R) was used with an octadecylsilica guard column (Resolve™ C18, Waters, MA, U.S.A.).

Creatinine, BUN, GGT and GPT Measurements Serum creatinine and BUN levels were determined with commercial kits (Wako Pure Chemicals Co., Ltd., Osaka, Japan) which employed the modified Jaffe’s reaction and urease-glutamate dehydrogenase method, respectively. Serum glutamate-oxaloate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) levels were determined with commercial kits (Wako Pure Chemicals Co., Ltd., Osaka, Japan).

Calculations A calibration curve was obtained for the amount of each amino acid against the peak area of respective amino acid derivative. The relative amount of D-amino acid in the total amount of the corresponding amino acid was calculated from the following equation:

\[ \%D = \frac{[D/(D + L)]}{100} \]

Differences between the groups were determined by the Student’s t-test for unpaired comparison.

RESULTS AND DISCUSSION

In order to investigate the correlations between D-Ala concentration in serum and the degree of renal dysfunction, concentrations of creatinine and BUN were also determined in the sera of 20 patients and of five normal...
subjects. As shown in Fig. 1, the D-Ala concentration in serum correlated positively with those of both creatinine ($r = 0.733$, $p < 0.001$) and BUN ($r = 0.449$, $p < 0.05$). The former correlation coefficient was consistent with the value obtained by Nagata et al. ($r = 0.832$, $p < 0.05$), who compared the total amounts of D-amino acids (D-Ala, D-Ser and D-Pro) with the concentration of creatinine.\(^1\)

The serum D-Ala may originate from intestinal bacteria since D-Ala is a constituent of the peptidoglycan layer of bacterial cell walls.\(^1\) The absorption rate of D-Ala from the gastrointestinal tract to the portal vein is considered to be similar between the patients and the normal subjects. However, the excretion of D-Ala into urine may be reduced owing to a low glomerular filtration rate in the patients. In addition, the metabolic conversion of D-Ala to pyruvic acid may be also decreased in the patients due to damage to the proximal tubular cells, where the highest activity of D-amino acid oxidase exists.\(^2\) The content of D-Ala in serum is therefore assumed to reflect renal dysfunction in glomerular filtration as well as in proximal tubular cells, which accounts for the correlation of D-Ala concentration to both creatinine and BUN levels. On the other hand, no correlation was observed between the serum level of D-Ala and the activity in serum of GOT or of GPT, which is used as a clinical marker for hepatic dysfunction. Thus, the serum D-Ala concentration is presumed to reflect renal disorders rather than hepatic dysfunction.

As also shown in Fig. 1, the L-Ala content in serum correlated negatively with creatinine ($r = 0.498$, $p < 0.05$) and BUN content ($r = 0.601$, $p < 0.01$), respectively, and the %D of Ala correlated better with creatinine ($r = 0.811$, $p < 0.001$) than BUN ($r = 0.559$, $p < 0.05$). It is presumed that damage to the proximal tubular cells also reduces the reabsorption of L-Ala and decreases L-Ala content in serum,\(^3\) resulting in an increase in the %D of Ala in serum.

The D-Ser concentration correlated positively with creatinine and BUN values as shown in Fig. 2. The correlation coefficient was 0.634 against the concentration of creatinine and 0.629 against that of BUN ($p < 0.001$). These results were consistent with the report of Bruckner.
et al., who suggested that the D-Ser in serum may be the indicator for renal dysfunction \((r = 0.667\) against creatinine). L-Ser concentration decreased with an increase in creatinine and BUN \((r = 0.493\) and 0.500, respectively, \(p < 0.05\)). The \(\%D\) of Ser correlated better with the concentration of BUN \((r = 0.919, p < 0.001)\) than that of creatinine \((r = 0.796, p < 0.001)\). It is known that serum levels of BUN are affected by protein turnover or catabolism in the tissues in addition to renal glomerular filtration. Although D-Ser has been found in rat and human brain, its origin in serum has not been clarified. The better correlation of the D-Ser concentration with the serum BUN suggests that D-Ser in serum originates in part during tissue protein catabolism. In addition, intra-peritoneal administration of D-Ser was reported to induce proximal tubular necrosis in rat kidney. The serum level of D-Ser may therefore reflect increased tissue catabolism as well as resulting renal dysfunction.

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REFERENCES