Cation-Exchange Chromatography of Guanidine Derivatives in Plasma of Patients with Chronic Renal Failure

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Guanidine derivatives are suspected of contributing to the toxic manifestations of uremia. We describe a method for measurement of guanidine derivatives in 5-ml samples of plasma by liquid chromatography. Concentrations of guanidinosuccinate and guanidinobutyrate in plasma were significantly increased both in undialyzed patients with chronic renal failure (5.54 ± 0.94 and 17.5 ± 4.07 mg/liter) and those undergoing maintenance hemodialysis (2.35 ± 0.41 and 19.4 ± 3.99 mg/liter) when compared to healthy controls (<0.4 and 1.0 ± 0.3 mg/liter, respectively). Creatine and guanidinoacetate concentrations tended to be higher in hemodialysis patients and lower in the undialyzed group of patients with chronic renal failure. This procedure provides a rapid, sensitive, and accurate method for the study of guanidine metabolism in persons with uremia.

Guanidine derivatives are suspected of contributing to the toxic manifestations of uremia. For example, methylguanidine intoxication in experimental animals produces a symptom complex similar to the uremic syndrome (1) and guanidinosuccinic acid has been implicated in the qualitative platelet defect responsible for uremic bleeding (2). Methodological limitations, however, have hindered attempts to isolate and quantify guanidines in biological fluids.

This report describes a high-pressure liquid chromatographic system for simultaneous determination of guanidine derivatives. We have measured the plasma concentration of these compounds in healthy volunteers, in patients with far-advanced chronic renal failure, and in patients receiving maintenance hemodialysis.

Materials and Methods

Analytical Methods

The compounds under study were separated by cation-exchange column chromatography. Twenty-five grams of cation-exchange resin (Aminex A-5; particle size, 13 ± 2 μm; BioRad Laboratories, Richmond, Calif. 94804) was washed in 250 ml of sodium citrate buffer (0.2 mol/liter, pH 3.25), stirred for 30 min, filtered through a fritted-glass funnel, and suspended again in fresh buffer. The resin was then packed into a 0.9 × 23 cm water-jacketed column (Beckman Instruments, Inc., Spinco Division, Palo Alto, Calif. 94304). The column was packed to a height of 19 cm at 58 °C (Lauda circulating water bath, Model K-2, Arthur Thomas Co., Philadelphia, Pa.), at 2020 kN/m² (20 atm) (multi-speed transmission pump, Model 600-000; Harvard Apparatus Co., Dover, Mass. 02030). After running 100 ml of the sodium citrate buffer at 40–60 ml/h at the same temperature and pressure, the column was ready for use.

Before chromatography, the proteins in a 5-ml sample of plasma were precipitated with 1.4 ml of a 200 g/liter solution of trichloroacetic acid, and the mixture was centrifuged (2000 rpm, 1 h). The decanted supernate was extracted three times with an equal volume of diethyl ether to remove the trichloroacetic acid, and the pH of the sample was adjusted to 5.5 ± 0.5 with sodium hydroxide 0.2 mol/liter. The sample (5 ml) was then applied to the column. It was eluted in 6 h with 160 ml of a sodium citrate buffer of increasing pH (3.25, 80 ml; 4.25, 40 ml; 5.26, 40 ml), followed by 40 ml of sodium hydroxide (0.2 mol/liter). The effluent was collected in 4-ml fractions with a fraction collector with a drop-counting device, and duplicate samples of the fractions were analyzed colorimetrically by a modification of the Voges–Proskauser reaction (3).

Chromatographically pure standards of arginine, guanidinosuccinic acid (Schwarz/Mann, Orangeburg, N. Y. 10962), guanidinoacetic acid, creatine (Sigma Chemical Co., St. Louis, Mo. 63178), and guanidinobutyric acid (Calbiochem, Los Angeles, Calif. 90059) were chromatographed to establish their elution patterns. Their concentrations were determined from calibration curves for these compounds in concentration of 5, 10, 15, 20, and 25 mg/liter. Analytical recoveries were determined after adding 55–125 μg of each compound to 5 ml of plasma. The coefficient of variation for replicate determinations was calculated. The partition coefficients for the various guanidine compounds, between ether and a trichloroacetic acid solution, were also determined.

Experimental Subjects

Eight hospitalized patients on maintenance hemodialysis, and five ambulatory patients with chronic renal failure not requiring hemodialysis were the subjects. Controls were six apparently healthy labo-
Table 1. Concentrations of Guanidine Derivatives in Plasma

<table>
<thead>
<tr>
<th>Group</th>
<th>Guanidinosuccinic acid</th>
<th>Guanidinoacetic acid</th>
<th>Guanidinobutyric acid</th>
<th>Arginine</th>
<th>Creatinine</th>
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<tr>
<td>Controls (C) (n = 6)</td>
<td>3.82 ± 0.40 (2.12 - 4.85)</td>
<td>0.35 ± 0.06 (0.18 - 0.59)</td>
<td>1.02 ± 0.32 (0.12 - 1.93)</td>
<td>14.4 ± 1.20 (11.9 - 19.2)</td>
<td>10 ± 3 (7 - 16)</td>
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<tr>
<td>Renal failure (RF) (n = 5)</td>
<td>5.54 ± 0.94 (3.10 - 110.7)</td>
<td>0.75 ± 0.23 (0.20 - 1.44)</td>
<td>17.5 ± 4.07 (4.70 - 25.9)</td>
<td>11.5 ± 1.47 (8.23 - 16.2)</td>
<td>120 ± 10 (80 - 150)</td>
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<tr>
<td>Hemodialysis (H) (n = 8)</td>
<td>2.35 ± 0.41 (0.66 - 96.5)</td>
<td>1.45 ± 0.48 (0.35 - 3.74)</td>
<td>19.4 ± 3.99 (6.60 - 38.3)</td>
<td>28.2 ± 4.68 (14.7 - 55.8)</td>
<td>150 ± 10 (110 - 170)</td>
</tr>
</tbody>
</table>

Group differences
- H vs. RF P < 0.018<sup>a</sup> NS NS NS P < 0.02 0.05 < P < 0.1
- H vs. C P < 0.001<sup>b</sup> NS 0.05 < P < 0.08 P < 0.005 P < 0.05 P < 0.001
- C vs. RF P < 0.004<sup>b</sup> NS NS P < 0.005 NS P < 0.001

Values are mean ± SE and (range); NS, P > 0.05.
<sup>a</sup> The patients with serum values of 110.7 and 96.5 mg/liter had clinical evidence of muscle wasting.
<sup>b</sup> Mann–Whitney U test.

Results

Figure 1 illustrates a typical chromatographic separation of a synthetic mixture of arginine, creatine, guanidinoacetate, guanidinosuccinate, and guanidinobutyrate. In this system, urea was eluted in fractions 4–6 and creatinine in fractions 33–35. Although its elution pattern overlapped somewhat with guanidinobutyrate, creatinine did not interfere with the Voges–Proskauer color reaction in the range of concentrations measured in our patients.

The analytical recovery of added known amounts of guanidines from plasma ranged from 87 to 102%. Experiments designed to measure the partition coefficient of the various guanidine derivatives between diethyl ether and a trichloroacetic solution revealed that less than 1% of each of the compounds under study was extracted into the ether layer. The coefficient of variation for eight replicate determinations of each compound ranged from 3 to 7%. In all cases the individual result ±2 SD overlapped with the corresponding duplicate analysis ±2 SD. The lower limit of sensitivity of the method ranged from 0.1 to 0.4 mg/liter.

The mean plasma concentration of each guanidine compound in control subjects and the two groups of patients is shown in Table 1. Except for arginine in those patients not undergoing hemodialysis, all values were higher in patients than in controls, although not statistically significantly so in the case of creatine and guanidinoacetate. The wide range in plasma creatine concentrations was largely attributable to above-normal values (96.5 and 110.7 mg/liter) found in two patients with marked muscle wasting.

<sup>1</sup> Signed informed consent was obtained from each subject and the protocol was approved by the Committee on Human Experimentation of the Miami Veterans Administration Hospital. The research was done according to the principles outlined in the Declaration of Helsinki.
Guanidinosuccinate concentrations in all the controls were <0.4 mg/liter, the lower limit of sensitivity of the test. When plasma concentrations of guanidinoacetate, guanidinobutyrate, or guanidinosuccinate in all subjects were grouped together and correlated with serum creatinine concentrations, a statistically significant relationship was obtained (P < 0.05, P < 0.001, and P < 0.05, respectively).

The chromatographic elution pattern for uremic plasma was similar to that for normal persons except for the presence of a small unidentified Voges–Proskaucer-positive peak corresponding to fractions 31–33. In one patient with combined renal and hepatic insufficiency the concentrations of arginine (8.2 mg/liter) and guanidinosuccinate (2.4 mg/liter) were much lower than was true of the patients with uncomplicated chronic renal failure and comparable serum creatinine concentration.

Discussion

We have described a rapid, sensitive, and accurate method for measuring guanidine derivatives in plasma and confirmed previously reported data on these compounds in the serum of healthy subjects and of patients with chronic renal insufficiency (1, 5).

Concentrations of guanidinosuccinate, guanidinoacetate, guanidinobutyrate, and creatine were greater in both groups of patients than in the healthy controls, although not statistically so in the case of guanidinoacetate and creatine. Arginine values tended to be higher in hemodialysis patients and lower in undialyzed renal failure patients.

Arginine values for the patients with chronic renal failure were comparable to those of normal controls (6, 7). The above-normal values found pre-dialysis in our hemodialysis patients may be a result of their higher protein intake or perhaps to breakdown of arginine conjugates during sample deproteinization or chromatography at low pH.

In a previous study in patients with chronic renal failure, Cohen (6) found values similar to ours for creatine in plasma but much greater concentrations of guanidinoacetate. He postulated that increased values for creatine and guanidinoacetate, caused by renal retention, inhibited L-arginine:glycine amidinotransferase (EC 2.1.4.1) with resulting transfer of the amidino group of arginine to aspartate to form guanidinosuccinate.

The presence of increased amounts of guanidinosuccinate in uremic serum has been reported (5, 8); in general, the concentrations we found in our patients are lower. Because guanidinosuccinate in plasma is decreased by hemodialysis (9), more efficient and more frequent dialysis in our subjects may explain this difference. Likewise, the use of protein-restricted diets may be responsible for the lower guanidinosuccinate values in our undialyzed patients with chronic renal failure (8).

Shainkin et al. (10) described a method for simultaneous measurement of guanidine derivatives in biological fluids by use of a modified amino acid analyzer. They found values for guanidinosuccinate similar to ours, but their values for guanidinoacetate and guanidinobutyrate were lower than in our cases. On the other hand, they found increased concentrations of guanidinopropionate in plasma of both dialyzed and undialyzed patients with chronic renal failure. Methylguanidine concentrations, previously reported to be increased in uremia (11, 12), were not different from controls. The practical advantage of the present method over that of Shainkin et al. (10) lies in its greater simplicity and perhaps lower cost of purchase and operation.

Although the exact role of these compounds in the production of uremic symptoms is difficult to define, it is likely that several guanidine compounds contribute to the toxicity of uremia (1, 6). The application of new analytical techniques for the measurement of these compounds in uremic patients is likely to increase our understanding of guanidine metabolism in chronic renal failure.

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References