Plasma Methylguanidine and Creatinine Concentrations in Cats with Experimentally Induced Acute Renal Failure

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ABSTRACT. Plasma methylguanidine (MG) and creatinine (CRN) concentrations were measured in 11 cats with experimentally induced acute renal failure by a two-stage surgical procedure. According to the progression of renal failure, both plasma MG and CRN levels increased. A significant positive correlation (y=0.187X−0.379, r=0.9176, P<0.001) was found between plasma MG and CRN levels. These results suggested that the increase in plasma MG level was an available indicator for uremic status in cats.—KEY WORDS: feline methylguanidine, renal failure.

It has been widely accepted that protein catabolism is a major feature of the end stage of renal failure [5]. Since the accumulation of some intermediate or terminal products from protein catabolism plays an important role in the development of the toxicity, the elimination of those accumulating substances from body fluids is essential for improvement of the uremic syndrome. Among those substances, methylguanidine (MG) has been paid particular attention, because the remarkable high serum MG concentration was detected in uremic, but not in non-uremic men, rats, and dogs with renal failure [1, 2, 8, 13].

The incidence of renal failure in cats, especially chronic renal failure, seems to be higher than that in other mammalian species [12]. In cats, the minimal protein requirement is considerably higher than that in other species. Higher protein intake may induce the higher generation rate of protein metabolites, which results in higher MG production. Therefore, cats might be more susceptible for renal failure and uremic status.

However, there are few reports on plasma MG levels in cats with renal failure. This note deals with plasma MG and creatinine (CRN) concentration in cats with experimentally induced acute renal failure.

Five male and 6 female cats (Ico: Fec Eur (Tif)) of weighing 1.5–3.8 kg and aged 1–3 years were used in this study. All cats were in good health on physical examination, complete blood cell counts and blood chemistry. To induce acute renal failure, nephrons of the cats were reduced by one eighth using a two-stage aseptic surgical procedure under anesthesia with medetomidine (100 μg/kg, I.M.) and ketamine (10 mg/kg, I.M.). At first, approximately three-fourths of the left kidney was infarcted by selective ligations of the renal arterial branches. Two weeks later, the right kidney was surgically removed under the same anesthesia. After the first surgery, cats were given water and commercial diet ad libitum and administered antibiotics (ampicillin, 30 mg/kg/day, I.M.) during the experimental period. Heparinized blood samples were obtained from all the cats just before the first surgery and then daily after the second surgery. When uremic symptoms of the experimental cats became serious, euthanasia was performed by overdosing of pentobarbital sodium.

The plasma was deproteinized by addition of trichloroacetic acid at a final concentration of 10%. The supernatant obtained by centrifugation at 3,000 rpm for 20 min was used for the analysis of MG. The concentration of MG was determined by the method of Hiraga and Kinoshita [6] with a slight modification using a high performance liquid chromatographic system (LC-6A system, Shimadzu, Japan) and ninhydrin as postcolumn fluorescent reactor. An ISC-05/S0504 packed column (strong cation-exchange resin; 5 μm particle; 38 mm × 4.6 mm I.D.; Shimadzu, Japan) with heating jacket maintained at 50°C was used for the separation. The mobile phase was citrate buffer (pH 11.4) consisted of 0.35 M sodium citrate, 0.65 M sodium chloride and 0.1 M boric acid. The column effluent was first mixed with 0.5 N sodium hydroxide and then mixed with 0.6% ninhydrin solution. The flow rates of the eluent solution, alkaline solution and ninhydrin solution were 0.7, 0.6 and 0.4 ml/min, respectively. The fluorescence intensity of the effluent was measured using an RF-535 LC spectrophotofluoromonitor (Shimadzu, Japan) at wavelengths of excitation (390 nm) and emission (470 nm). The lower limit of this MG assay system was approximately 0.05 nmol/ml. Plasma concentrations of CRN were measured by alkaline picate method using an automatic chemical analyzer (COBAS-MIRA, Japan Roche).

Plasma MG was not detectable in all the preoperative cats. After the second surgery, plasma MG level increased in all the cats and the highest levels (0.242–5.244 nmol/ml) were recorded at just before euthanasia (Table 1). Plasma CRN levels also increased after second surgery. Figure 1 shows the relationship between MG and CRN levels determined in the cats during the experiment. A significant positive correlation was found between plasma MG (y) and CRN (x) values with a linear regression equation (y=0.187X−0.379, r=0.9176, P<0.001). A significant positive linear regression (y=0.224X−0.890, r=0.9303, P<0.001) with a higher gradient was observed in the data with CRN levels more than 5 mg/dl, when the uremic status was severer.

Many investigators reported that MG concentrations of body fluids increased in animals with uremic status [1, 2, 8,
Table 1. Plasma creatinine and methylguanidine (mean ± SD) levels in 11 cats with experimentally induced acute renal failure

<table>
<thead>
<tr>
<th>Day after operation</th>
<th>No.</th>
<th>Creatinine Mean</th>
<th>Creatinine SD</th>
<th>Methylguanidine Mean</th>
<th>Methylguanidine SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>1.35</td>
<td>0.553</td>
<td>0.136</td>
<td>0.052</td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>3.84</td>
<td>2.370</td>
<td>0.181</td>
<td>0.075</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>5.34</td>
<td>3.066</td>
<td>0.267</td>
<td>0.152</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>4.55</td>
<td>1.115</td>
<td>0.399</td>
<td>0.162</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>7.28</td>
<td>3.551</td>
<td>1.084</td>
<td>0.487</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>14.05</td>
<td>6.521</td>
<td>2.280</td>
<td>1.492</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>18.81</td>
<td>6.180</td>
<td>3.447</td>
<td>1.759</td>
</tr>
</tbody>
</table>

13]. In addition, MG treatment also induced the clinical symptoms similar to those observed in uremic patients [4, 7]. In this study, plasma MG levels markedly increased in cats with experimentally induced acute renal failure.

The route of MG production has been investigated in vitro. Creatinine, arginine and guanidinoacetic acid were considered to be the probable precursors of MG [3, 11]. Recently Nakamura et al. [10] reported that MG was synthesized through creatol (5-hydroxy-creatine) from CRN in vitro and suggested that CRN may be one of the precursors of MG. In this study, a significant positive correlation was found between plasma MG and CRN values. Moreover, the gradient of a linear regression equation in the data with plasma CRN values more than 5 mg/dl was higher than that in the all data during the experimental period. Those results suggest that MG in the cat with acute renal failure might be synthesized from CRN in vivo, however, the production of MG may depend on other factors including pH of body fluids, circulating blood volume, and active oxygen [9]. Further study will be needed to clarify the mechanism of MG production in cats.

In conclusion, a significant amount of MG was produced in cats with acute renal failure and a significant correlation between the plasma MG and CRN levels was observed. Thus, it was suggested that plasma MG level was an available indicator of uremic status in cats.

REFERENCES


Fig. 1. Correlation between plasma methylguanidine and creatinine levels in cats with experimentally induced acute renal failure. Solid line (γ=0.187X – 0.379, r=0.9176, p<0.001) represents the linear regression equation on the all data and dashed line (γ=0.224X – 0.890, p=0.903, p<0.001) represents the linear regression equation on the data with creatinine values more than 5 mg/dl, respectively.