Interrelation of Urinary and Plasma Levels of Guanidinoacetic Acid with Alteration in Renal Activity of Glycine Amidinotransferase in Acute Renal Failure Rats

Yukihito KUWAGAKI and Jun-ichi SUDO*

Department of Toxicology and Clinical Pharmacology, Faculty of Pharmaceutical Sciences, Higashi-Nippon-Gakuen University, Ishikari-Tobetsu, Hokkaido 061-02, Japan. *Received July 30, 1988*

The present study was undertaken to investigate the changes of plasma and urinary levels of guanidinoacetic acid (GAA) in relation to the alteration of renal activity of glycine amidinotransferase (GAT) in the acute stage of renal failure. Rats received cephaloridine at doses of 0 (control), 100 and 1000 mg/kg body weight. The 100 mg/kg group showed rises in the urinary excretion of GAA from the 2nd to the 4th day, but did not show any changes in the other items determined. The urinary excretion of GAA in the 1000 mg/kg group showed a rise on the 1st day, and a fall on the 3rd day. The renal arginine in the group fell from days 1 to 4. The renal activity of GAT in the group fell from day 2, reached the lowest level on day 3, and reverted to the control level after day 5. These results suggest that the rise in the urinary excretion of GAA on the 1st day was ascribable to an inhibitory effect of cephaloridine on renal reabsorption of GAA, and that the fall in its urinary excretion on the 3rd day was ascribable to the suppression in the renal GAA formation system including GAT, arginine and so on.

Keywords glycine amidinotransferase; guanidinoacetic acid; arginine; creatine; creatinine; cephaloridine; urine; kidney; toxicity; rat

Introduction

Guanidinoacetic acid (GAA) is an essential precursor of creatine, and is synthesized from glycine and arginine by glycine amidinotransferase (GAT: EC 2.1.4.1) in the kidney, the pancreas and other organs.[1] The kidney is generally accepted to be the primary site of GAA synthesis, at least in animals.[2] In addition, GAA, an intermediate metabolite and precursor of creatine, is known to be excreted into urine, and it has been suggested as a possible index of nephropathy at the urinary and plasma levels.[3] However, the etiology of the decrease in the urinary excretion of GAA generally observed in the above nephropathy has remained to be investigated. Further, conflicting results on the plasma level of GAA were reported, and this conflict needs to be resolved.

We considered that the changes of GAA at urinary and plasma levels should be estimated in relation to the alteration of renal GAT activity. Thus, in the present study, we endeavored to investigate GAA metabolism mainly at the renal level, and additionally at the urinary and plasma levels, in rats with acute renal failure induced by cephaloridine (CER), which is a nephrotoxin acting on the renal proximal tubules.[4] Here, we present the results obtained.

Experimental

Chemicals Cephaloridine (CER) (Keflodin) was purchased from Shionogi Co. (Osaka, Japan), a standard mixture of guanidino compounds and creatinine from Wako Pure Chemical Co. (Osaka, Japan), and arginine HCl and glycine from Kanto Chemical Industries Co. (Tokyo, Japan). All other reagents used in this study were commercial products of the highest grade available.

General Procedures Male Wistar rats weighing 210—230 g (Sankyo Labo Service Co.; Tokyo, Japan) were used for this study, kept in ordinary cages, and allowed free access to water and standard diet pellet (MF; Oriental Yeast Co.; Chiba, Japan). In order to avoid diurnal changes, dosage and other operations to the rats were done between 10:00 a.m. and 11:00 a.m.[5a] The rats received single intravenous injections of CER in an equivalent volume of saline (4 ml/kg body weight) through the tail vein, and the doses were 100 and 1000 mg/kg body weight. The control rats received the same volume of saline. The time-point when the administrations were done, was designated as day 0. The period from 0 to 24 h following the administrations was designated as the 1st day.

Experimental Design Protocol 1: The control and CER-administered rats were singly placed in metabolism cages (KN-646, B-1 type; Natsume Co., Tokyo, Japan), in order to obtain urine for determinations of GAA and creatinine. The rats were given free access to water and diet pellet in the metabolism cages. Throughout 7 d, the urine was collected every 12 h in a flask in which 5 ml of water-saturated mineral oil and 50 μl of 0.5 mg/ml of sodium azide had been put beforehand. Other operations were done as reported previously.[4d]

Protocol 2: For the analyses of the kidney and plasma (see below), renal homogenate and plasma were prepared on days 1, 2, 3, 4, 5, 6 and 7 after the CER administration. Under ether anesthesia, the abdominal cavity was opened through a ventral incision. Blood was taken from the abdominal aorta, put into a heparinized tube, and centrifuged ($1700 \times g$, 10 min, 4 °C) for separation of plasma. The plasma was used for determinations of GAA, creatine and creatinine. After taking the blood, the left kidney was perfused through the abdominal aorta with saline, removed, weighed, and homogenized in a glass/Teflon Potter-Elvehjem homogenizer with approximately 8 ml of 0.25 M sucrose. In order to adjust the homogenate so that 10 ml contained one kidney, 0.25 M sucrose was further added. The resultant homogenate was used for measurements of renal contents of GAA and arginine, and of renal activities of GAT.

Analytical Procedures GAA, arginine, creatine and creatinine were determined by the high-performance liquid chromatographic (HPLC) method of Hiraga and Kinoshita[5b]: this method involves post-labeling with ninhydrin. For deproteinization, one volume of 60% (w/v) trichloroacetic acid was added to five volumes of sample. This mixture was centrifuged ($10062 \times g$, 10 min, 4 °C), and its supernatant was used for the above HPLC analysis. Quantities of GAA, arginine, creatine and creatinine were calculated from standard curves made using a standard mixture of guanidino compounds and creatine. Protein content in the renal homogenate was determined by the method of Kresze,[5c] based on the method of Lowry *et al.*[5d] Renal activity of GAT was spectrophotometrically determined by the method of Van Pilsum *et al*,[5e] using glycine and arginine as substrates, and was expressed as μmol of ornithine formed per min per g protein at 37 °C (μmol/min/g protein).

Statistics The results were represented as the mean ± S.E.M. To define statistically significant differences among the groups, the data were subjected to one-way analysis of variance and subsequently treated according to Bonferroni's method[5f]; p values of less than 0.05 were considered significant.

Results

In the first series of experiments of protocol 1, urinary excretions of GAA and creatinine were investigated for 7 d after CER administration at 0 (control), 100 and 1000 mg/kg.

Figure 1 shows the changes in the urinary excretion of GAA. The excretion in the 100 mg/kg group rose from the

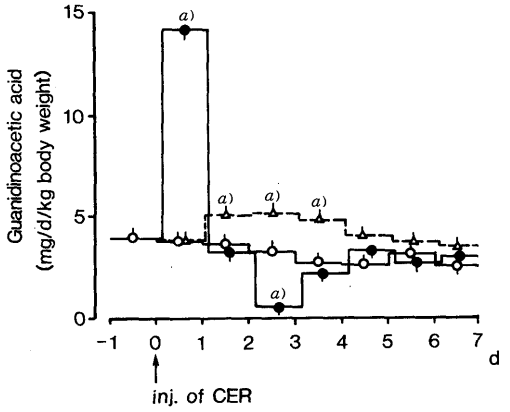

Fig. 1. Changes in Urinary Excretion of Guanidinoacetic Acid Following Single Intravenous Injection of Cephaloridine in Rats

Points and vertical bars represent the mean ± S.E.M. The number of rats was 6 in each group. Significant differences in comparison to the control group at each time point: *a)* $p<0.01$, *b)* $p<0.05$. Abbreviations: CER, cephaloridine; inj. of CER, injection of cephaloridine; ○—○, control, group administered saline; △—△, CER 100 mg/kg, group administered CER at the dose of 100 mg/kg body weight; ●—●, CER 1000 mg/kg, group administered CER at the dose of 1000 mg/kg body weight.

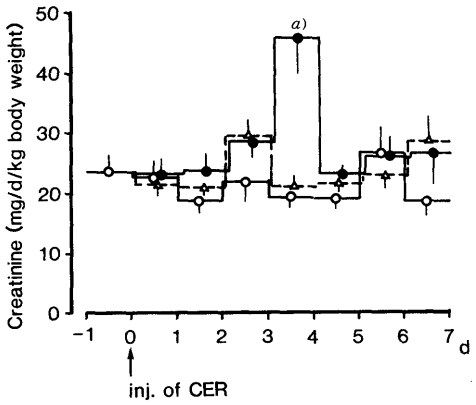

Fig. 2. Changes in Urinary Excretion of Creatinine Following Single Intravenous Injection of Cephaloridine in Rats

Explanations are as in Fig. 1.

2nd to the 4th day, and reverted afterward. The excretion in the 1000 mg/kg rose (3.5 times the control) on the 1st day, fell (20% of the control) on the 3rd day, and reverted to the control level after the 4th day.

Figure 2 shows the changes in the urinary excretion of creatinine. No significant difference between the control and the 100 mg/kg groups was observed. The excretion in the 1000 mg/kg group showed a rise (2.3 times the control) on the 4th day; at the other time-points, the excretion was within the control range.

In the second series of experiments of protocol 2, plasma concentrations of GAA, creatine, and creatinine, renal contents of GAA and arginine, and renal activity of GAT, were investigated at the time-points of days 1, 2, 3, 4, 5, 6 and 7 after CER administration, in the control and the two CER-administered groups. Since no significant difference was observed between the control and the 100 mg/kg group, the following section compares only the 1000 mg/kg group and the control group.

The plasma GAA in the 1000 mg/kg group showed a fall (35% of the control) on day 3, and a rise (1.5 times the control) on day 5 (Fig. 3). The plasma creatine in the group showed a fall (64% of the control) on day 3, and a rise (1.5

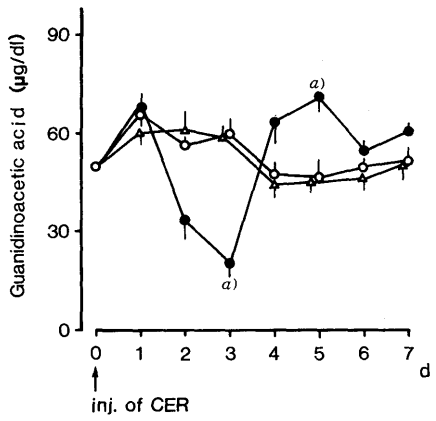

Fig. 3. Changes in Plasma Concentration of Guanidinoacetic Acid Following Single Intravenous Injection of Cephaloridine in Rats

The number of rats was 6 at each time point. Other explanations are as in Fig. 1.

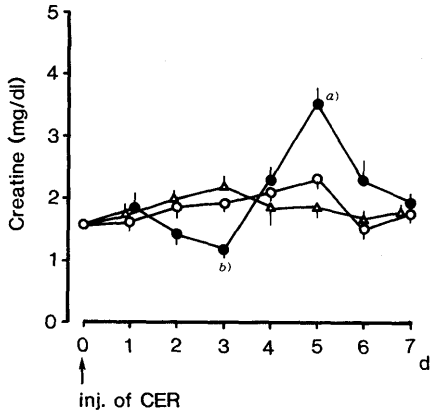

Fig. 4. Changes in Plasma Concentration of Creatine Following Single Intravenous Injection of Cephaloridine in Rats

Explanations are as in Fig. 3.

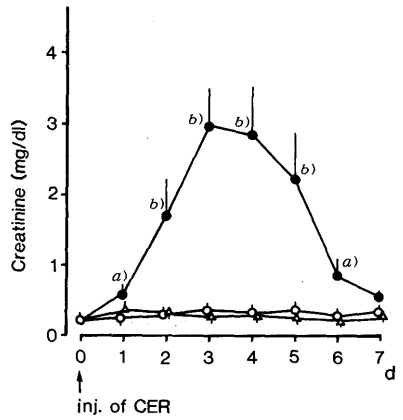

Fig. 5. Changes in Plasma Concentration of Creatinine Following Single Intravenous Injection of Cephaloridine in Rats

Explanations are as in Fig. 3.

times the control) on day 5 (Fig. 4). The plasma creatinine in the group began to rise from day 1, reached a maximum (8 times the control) on day 3, and approached the control level on day 7 (Fig. 5).

The renal GAA in the 1000 mg/kg group rose (1.5 times the control) on day 1, fell (40% of the control) on day 3, and reverted to the control level afterward (Fig. 6). The renal arginine in the group fell (71 to 73% of the control) from

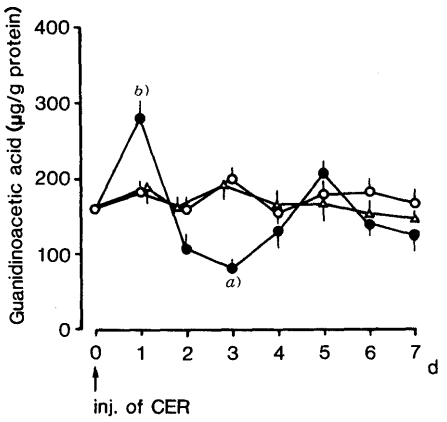

Fig. 6. Changes in Renal Content of Guanidinoacetic Acid Following Single Intravenous Injection of Cephaloridine in Rats

Explanations are as in Fig. 3.

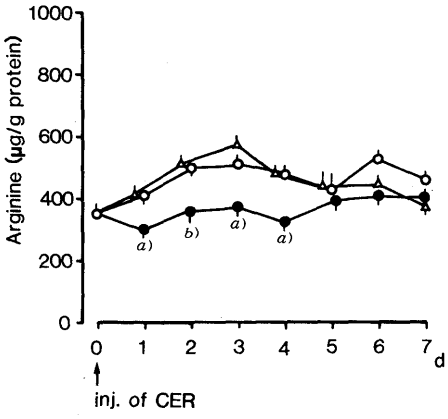

Fig. 7. Changes in Renal Content of Arginine Following Single Intravenous Injection of Cephaloridine in Rats

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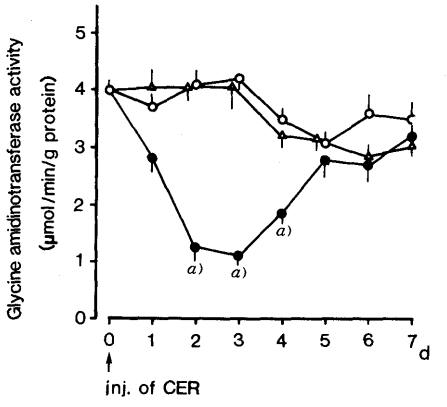

Fig. 8. Changes in Renal Activity of Glycine Amidinotransferase Following Single Intravenous Injection of Cephaloridine in Rats

Explanations are as in Fig. 3.

days 1 to 4, and reverted to the control level afterward (Fig. 7). The renal activity of GAT in the group fell from day 2, reached the lowest level (27% of the control) on day 3, and reverted to the control level after day 5 (Fig. 8).

Discussion

In recent years, considerable attention has been paid to the metabolic changes of GAA that occur in renal failure.[3,6] Most of those researches have dealt with the chronic stage of renal failure. As described in the introduction, decreases in the urinary excretion of GAA were commonly observed. In contrast, the results on the plasma concentration of GAA were inconsistent with each other: a decrease was in some cases, and increases in other cases. This inconsistency may have arisen from alterations in GAT activity as well as in the ability of the injured kidney to excrete GAA in the urine, obscuring the alteration of GAA at the urinary and plasma levels. Adaptation to the impaired GAA metabolism in chronic renal failure may have been more or less accomplished. In order to exclude the latter possibility, and to obtain information on the time course of changes in GAA metabolism, we selected as an experimental model not the above chronic renal failure, but the acute renal failure induced by CER, and endeavored to elucidate the changes of GAA and GAT in the kidney.

Furthermore, in terms of renal metabolism of glycine and arginine, precursors of GAA, it is known that the kidneys trap glycine, which is derived from the peripheral metabolism of amino acids, and convert glycine into serine and GAA,[7] and that, in contrast, arginine is synthesized in the kidneys.[8] Also, regarding the blood levels of amino acids in rats with chronic renal failure, it has been reported that, though the arginine level fell, the glycine level remained unchanged.[6f] These findings suggest that in respect to GAA synthesis in renal failure, arginine may be more important than glycine. Thus, alterations in the renal arginine synthesis were also observed in this study.

The nephrotoxin CER produces renal proximal tubular injury in humans and laboratory animals.[4a-c,9] In rats, GAT distributed in the proximal tubules[10] (in the inner membrane of mitochondria of proximal tubules[10b]). Further, arginine is synthesized from citrulline, an intermediate of the urea cycle, by argininosuccinate synthetase and argininosuccinase, both of which are located in the kidney[8] (argininosuccinate synthetase is present in the cytoplasma of the proximal tubules[8c]). Concerning the doses adopted in this study, the LD_{50} value in rats was 1.3 to 1.4 g/kg body weight in single intravenous administration,[9] and the ND_{50} (nephrotoxic dose) value was 1.0 g/kg body weight in single subcutaneous administration.[9a] Based on those and other reports,[11] the doses of CER and periods for sampling were selected.

In this study, creatinine levels in blood and urine were determined for estimation of the degree of CER-induced nephrotoxicity. In the 1000 mg/kg group, the blood level was maximal on the 3rd day (Fig. 5), and the urinary level on the 4th day (Fig. 2). This result indicated that the degree of injury was maximal on the 3rd day; the peak of urinary creatinine on the 4th day was considered to reflect the excretion of blood creatinine into urine as the renal injury was repaired.

In addition, our results revealed that the urinary excretion of GAA in the 100 mg/kg group rose from the 2nd to the 4th day (Fig. 1). The rises appeared to correspond to those in the 1000 mg/kg group on the 1st day, showing a retardation and a moderate intensity as compared with the 1000 mg/kg group. The other items determined in the 100 mg/kg group were within the control range throughout the whole process, whereas the 1000 mg/kg group showed significant changes in comparison to the control group. The following discussion will therefore deal mainly with the

results obtained from the 1000 mg/kg group.

Our main finding is that the renal activity of GAT was depressed from days 2 to 4 (Fig. 8), and that renal content of arginine was also diminished from days 1 to 4 (Fig. 7). These findings indicate that CER damaged the systems concerned with the GAA and arginine formation in the proximal tubules. CER might also impair the proximal tubular trapping system for arginine. For one or more of these reasons, GAA formation would be suppressed in the proximal tubules during this period (Fig. 6). This proximal tubular suppression of GAA formation could be a reason for the falls of GAA at the urinary, plasma and renal levels on day 3 (Figs. 1, 3 and 6), in good accordance with the precedents[6a,b] in the literature. However, the rises of GAA at the urinary and renal levels on day 1 (Figs. 1 and 6) could not be explained by the above mechanism.

Concerning renal transport of GAA, Kadano *et al.*[12a] reported that GAA was secreted into urine in the case of a high plasma level of creatine, whereas it was reabsorbed in the case of a low plasma level of creatine. We found no significant change of plasma creatine on day 1 (Fig. 4). Accordingly, it seemed unlikely that the plasma creatine had affected renal transport of GAA. As a possible cause of the above rise of GAA at the urinary level on the 1st day, a toxic effect of CER on the renal transport would be considered. It is known that CER damages the proximal tubules, and that physiological dysfunction of secretion and reabsorption systems for a variety of substances would subsequently occur[4a,d,12b]: in this situation, the GAA would be less effectively reabsorbed in the proximal tubules, and the urinary excretion of GAA would be increased. To account for the rise in renal content of GAA on day 1, the following two factors were considered: firstly, CER might cause cellular accumulation of GAA due to damage to the urinary secretion mechanism of GAA, and secondly the renal formation of GAA might be promoted through an unknown mechanism(s).

In addition, our results revealed that the levels of the plasma GAA and creatine in the 1000 mg/kg group were elevated on day 5 (Figs. 3 and 4). This finding might be explained as follows. In order to compensate for the depression of renal GAA production on day 3, production of GAA and creatine in other organs than the kidney might be promoted from days 3 to 5.[13] On day 5, renal GAA production reverted to the control level. At day 5, the plasma levels of GAA and creatine thus reflected the normal production in the kidney plus the overproduction in the other organs, and were consequently elevated.

Finally, we will discuss the usefulness of GAA and creatinine for evaluation of nephropathy. Our study suggests that GAA at the urinary level could be the most sensitive and earliest index for evaluation of renal injury, at least under our experimental conditions. However, even when the urinary excretion of GAA is within the normal range, it is uncertain whether this state is normal or not. The result in the 1000 mg/kg group on the 2nd day illustrates this (Fig. 1): the urinary excretion of GAA was normal, although renal arginine and GAT, and plasma creatinine clearly revealed the presence of renal injury (Figs. 5—8). Thus, when the urinary excretion of GAA is utilized as an index for evaluation of renal injury, especially in the acute stage of renal failure, the results needs to be interpreted from the viewpoints of both renal GAA formation and renal GAA transport.

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