Guanidino Compounds in Serum and Urine of Cirrhotic Patients

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To investigate the metabolic relationship between urea and guanidinosuccinic acid (GSA), we determined the levels of the guanidino compounds, including GSA, and urea in serum and urine of cirrhotic patients. Linear correlation studies between serum urea and GSA levels were performed. Good positive linear correlation coefficients were found in the Child-Turcotte C subgroup ($r = .847$, $P < .001$) and in the total subgroup including B and C patients ($r = .848$; $P < .0001$). Serum guanidinoacetic acid levels were significantly increased in the Child-Turcotte C subgroup ($P < .0001$ for men and $P < .001$ for women). In contrast, GSA levels were significantly ($P < .0001$) decreased in the three studied subgroups. Similar results were found for urinary GSA excretion levels. Within each subgroup, serum and urinary GSA levels were significantly lower in patients with alcohol-induced cirrhosis than in nonalcoholic cirrhotic patients. Similar results were obtained for urea. The findings in cirrhotic patients clearly demonstrate a metabolic relationship between urea and GSA. They also show that urea and GSA biosynthesis is significantly lower in cirrhotic patients with an alcoholic origin than in cirrhotic patients with a nonalcoholic origin.

ONE OF THE MAIN disturbed metabolic systems in cirrhotic patients is certainly ammonia nitrogen metabolism. Indeed, in patients with severe liver disease high ammonia levels are frequently found and not easy to decrease, since the cirrhotic liver fails to metabolize ammonia by the urea cycle.[1] Therefore, the capacity for urea synthesis is reduced and the daily urea nitrogen synthesis rate is significantly decreased in cirrhotic patients.[2,3]

The pathobiochemistry of the guanidino compounds in uremia and hyperargininemia has shown a metabolic relationship between urea and guanidinosuccinic acid (GSA).[4] Two hypotheses for the biosynthesis of GSA have been proposed. In 1970, Cohen suggested that GSA could be formed by transamination of arginine to aspartic acid.[5] According to this hypothesis, GSA would be a direct catabolite of arginine, formed through one enzymatic reaction. In 1979, Natelson and Sherwin[6] proposed an alternative hypothesis: GSA would be formed through different enzymatic steps from urea. The pathobiochemistry of GSA in uremia and hyperargininemia could support the last hypothesis: uremic patients, characterized by increased serum urea levels, are also characterized by increased serum GSA levels. Patients with hyperargininemia characterized by a disturbed urea cycle and decreased urea biosynthesis, certainly while undergoing treatment with protein restriction together with supplementation of essential amino acids with or without sodium benzoate, also display a decreased biosynthesis of GSA.[4]

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We performed this study to investigate whether the metabolic relationship between urea and GSA is also seen in cirrhotic patients. Therefore, we determined the levels of the guanidino compounds, including GSA, and urea in serum and urine of a cirrhotic population. Correlation studies were performed.

SUBJECTS AND METHODS

Patients

We studied 64 cirrhotic patients (37 men, 27 women) with varying degrees of liver failure and an age range of 30 to 79 years. The etiologies were alcoholism (n = 49), chronic active hepatitis (n = 9), Budd-Chiari syndrome (n = 1), and iatrogenic (n = 1). In addition, there were three cases of unknown origin and one case of primary biliary cirrhosis. Cirrhotic patients with renal insufficiency were not considered for this study. Diagnosis was based on history, clinical findings, biochemical data, and radiologic examinations, and in some cases, histologic data.

All cirrhotic patients were classified according to criteria reported by Child and Turcotte.[7] The following items were scored: serum bilirubin (mg/dL: $<2.0 = 1$, 2.0 to 3.0 = 2, and $>3 = 3$); serum albumin (g/dL: $>3.5 = 1$, 3.0 to 3.5 = 2, and $<3 = 3$); ascites (none = 1, easily controlled = 2, and poorly controlled = 3); encephalopathy (none = 1, minimal = 2, and advanced = 3); nutrition (excellent = 1, good = 2, and wasting = 3); and prothrombin time (1 second prolonged = 1, >1 to <4 seconds prolonged = 2, and 4 seconds prolonged = 3). Total scores of 6 to 9 corresponded to Child-Turcotte A, scores between 10 and 14 to B, and scores between 15 and 18, or more than 12 if coma, tense ascites, or substantial encephalopathy was present, to C.

Collection and Preparation of Samples

Fasting sampling was performed in the morning. After clotting, blood was centrifuged at 2,200 × *g* at 6°C for 10 minutes. A portion of the serum was reserved for urea determination. The remaining serum was stored at −75°C until analyzed. Fasting morning urine was also collected. For determination of the guanidino compounds, serum and urine samples were deproteinized by mixing equal volumes of a 200-g/L trichloroacetic acid solution with serum or urine. The proteins were centrifuged in a Beckman microfuge (Beckman Instruments International, Geneva, Switzerland). Two hundred microliters of supernatant was used for analysis.

Guanidino Compounds and Other Chemicals

Standard guanidino compounds were acquired from Sigma Chemical (St Louis, MO), and creatine and creatinine were from Merck (Darmstadt, Germany). α-Keto-δ-guanidinovaleric acid was synthesized enzymatically as described previously.[8] All other reagents were obtained from Merck and were of analytical grade.

Laboratory Methods

The concentration of guanidino compounds was determined using a Biotronik LC 5001 amino acid analyzer (Biotronik, Maintal, Germany) adapted for guanidino compound determination. Guanidino compounds were separated over a cation-exchange column using sodium citrate buffers and were detected with the fluorescence ninhydrin method as previously reported in detail.[8] Serum urea nitrogen was determined with diacetylmonoxime as described by Ceriotti.[9]

Results are presented as the mean ± SD for guanidino compounds present at detectable levels. If, next to detectable levels of a particular guanidino compound, no levels could be detected in some samples, then the results are given as a range from less than the detection limit to the highest level obtained in this group. In urine the levels of creatine have a large dispersion, and therefore in this case results were also expressed as a range from the lowest to the highest value. Results were compared using ANOVA with least-significant difference post hoc comparison (ANOVA, SPSS, Imara, Leuven, Belgium). In Table 3, Student's *t* test was used for comparisons between two groups. The interrelationship of individual serum GSA levels with corresponding serum urea nitrogen levels was assessed by linear correlation studies.

RESULTS

Levels of serum arginine and homoarginine in cirrhotic patients are not significantly different from control levels (Table 1). Serum levels of guanidinoacetic acid, the first component of the creatine-creatinine biosynthesis pathway, are significantly ($P < .0001$ for men and $P < .001$ for women) increased in Child-Turcotte C patients. Serum GSA levels are significantly ($P < .0001$) decreased in all studied cirrhotic patients independent of their Child-Turcotte score. Urea levels are also decreased, although to a lesser extent, in all cirrhotic patients. The decrease is significant in B and C subgroups.

As in serum, urinary GSA excretion levels are significantly decreased ($P < .01$ for A and $P < .0001$ for B and C subgroups) in all studied cirrhotic patients (Table 2). Urinary excretion of γ-guanidinobutyric acid is also significantly decreased.

Within each subgroup, serum and urinary guanidino compound levels of cirrhotic patients with alcoholic origin were compared with those of cirrhotic patients with nonalcoholic origin. This comparative study demonstrated that levels of GSA in both serum and urine were significantly lower in patients with alcoholic cirrhosis than in other patients (Table 3). Also, serum urea levels of alcoholic cirrhotic patients are significantly lower than those seen in nonalcoholic cirrhotic patients, except for the B subgroup,

Table 1. Serum Guanidino Compound (μmol/L) and Urea (mmol/L) Levels in Controls and Cirrhotic Patients Classified According to the Criteria of Child-Turcotte

Guanidino Compound	Controls (n = 66)	Child-Turcotte Subgroups		
		A (n = 29)	B (n = 22)	C (n = 13)
α-Keto-δ-guanidinovaleric acid	<0.035-0.200	<0.035-0.220	<0.035	<0.035-0.150
GSA	0.259 ± 0.096	0.139 ± 0.149§	0.118 ± 0.106§	0.097 ± 0.133§
Creatine				
Men	30.1 ± 12.3	29.8 ± 22.2	30.8 ± 27.4	16.5 ± 11.5
Women	54.8 ± 21.0	28.3 ± 16.2†	35.9 ± 30.7*	56.6 ± 50.2
Guanidinoacetic acid in				
Men	2.61 ± 0.517	3.10 ± 1.70	3.46 ± 1.44	5.20 ± 2.95§
Women	2.01 ± 0.572	2.67 ± 1.71	2.68 ± 1.24	4.36 ± 2.64‡
α-*N*-acetylarginine	<0.015-0.620	<0.015-0.420	<0.015-0.500	<0.015-0.600
Argininic acid	<0.015-0.440	<0.015-0.410	<0.015-0.500	<0.015-0.130
Creatinine				
Men	80.8 ± 17.7	56.5 ± 13.7§	56.0 ± 13.6‡	65.5 ± 18.3*
Women	65.3 ± 19.7	56.9 ± 15.0	64.5 ± 28.3	54.2 ± 19.8
γ-Guanidinobutyric acid	<0.013-0.055	<0.013-0.09	<0.013 -0.08	<0.013
Arginine	110 ± 23.9	105 ± 37.2	97.0 ± 29.1	98.7 ± 41.7
Homoarginine				
Men	1.98 ± 0.634	1.87 ± 0.788	1.65 ± 0.785	1.79 ± 0.670
Women	1.51 ± 0.609	1.52 ± 0.761	1.24 ± 0.382	1.62 ± 1.03
Guanidine	<0.06-0.210	<0.06-0.300	<0.06-0.350	<0.06-0.400
Urea	442 ± 1.10	4.04 ± 1.93	3.51 ± 1.53*	3.18 ± 2.06†

NOTE. Levels of β-guanidinopropionic acid and methylguanidine were lower than the detection limit, ie, <0.013 and <0.02 μmol/L, respectively. The control group had 33 men and 33 women, the A group 19 and 10, the B group 9 and 13, and the C group 8 and 5. Data were compared using ANOVA with least-significant difference post hoc comparison (ANOVA, SPSS). *P* values indicate significant differences from control levels.

*$P < .05$.

†$P < .01$.

‡$P < .001$.

§$P < .0001$.

Table 2. Urinary Guanidino Compound (μmol/g CTN) and Urea (mmol/g CTN) levels in Controls and Cirrhotic Patients Classified According to the Criteria of Child-Turcotte

Guanidino Compound	Controls (n = 34)	Child-Turcotte Subgroups A (n = 29)	B (n = 23)	C (n = 13)
α-Keto-δ-guanidinovaleric acid	<DL-35	<DL-35	<DL-35	<DL-35
GSA	25.0 ± 9.03	16.3 ± 13.9†	11.7 ± 8.52‡	9.87 ± 14.5‡
Creatine				
Men	30-1,700	43-1,240	14-4,720	25-170
Women	30-3,200	37-3,440	33-1,610	30-4,680
Guanidinoacetic acid	223 ± 128	336 ± 326	206 ± 201	205 ± 152
α-*N*-acetylarginine	22.2 ± 9.63	28.4 ± 13.7	23.9 ± 10.5	21.7 ± 9.32
Argininic acid	6.73 ± 2.80	6.44 ± 2.73	6.66 ± 5.22	5.33 ± 2.76
β-Guanidinopropionic acid	<DL-1.00	<DL-1.00	<DL-1.75	<DL-0.620
γ-Guanidinobutyric acid	11.8 ± 9.29	9.69 ± 7.04	7.51 ± 4.60*	4.14 ± 2.43†
Arginine	20.9 ± 15.9	14.9 ± 8.29	15.1 ± 7.18	17.4 ± 35.3
Homoarginine	<DL-6	<DL-1.4	<DL-2.4	<DL-15
Guanidine	9.75 ± 3.38	12.5 ± 7.73	12.0 ± 5.94	10.8 ± 4.23
Methylguanidine	2.99 ± 1.24	<DL-10	<DL-10	<DL-10
Urea	185 ± 48.6	189 ± 68.6	192 ± 93.6	145 ± 71.8

NOTE. The control group had 15 men and 19 women, the A group 19 and 10, the B group 10 and 13, and the C group 8 and 5. Data were compared using ANOVA with least-significant difference post hoc comparison (ANOVA, SPSS). *P* values indicate significant differences from control levels.

Abbreviation: CTN, creatinine; DL, detection limit.

*$P < .05$.

†$P < .01$.

‡$P < .0001$.

in which levels were only marginally significantly lower ($P = .054$). Urinary urea excretion levels found in patients with alcohol-induced cirrhosis were also marginally significantly lower ($P = .057$ in A subgroup and $P = .063$ in C subgroup) than those observed in nonalcoholic patients, except for the B subgroup.

Figure 1 shows the relation between serum urea levels of B and C patients and the corresponding levels of serum GSA. A significant positive linear correlation ($r = .848$, $P < .0001$) shows that patients with lower serum urea levels also had lower serum GSA levels.

DISCUSSION

The urea cycle incorporates nitrogen not required for net-biosynthetic purposes into urea, which serves as a waste nitrogen product in ureotelic animals. The urea cycle also forms part of those biochemical reactions required for de novo biosynthesis and degradation of arginine. In ureotelic animals, a large proportion of arginine biosynthesis takes place in the liver. Arginine and homoarginine, both hydrolyzed to urea by arginase, have normal serum and urinary excretion levels in our studied cirrhotic patients, thus suggesting that their arginine and homoarginine hepatic

Table 3. Serum GSA (μmol/L) and Urea (mmol/L) and Urinary GSA (μmol/g CTN) and Urea (mmol/g CTN) Levels in Alcoholic and Nonalcoholic Cirrhotic Patients Classified According to the Criteria of Child-Turcotte

	Child-Turcotte Subgroups					
	A		B		C	
	Alcoholic	Nonalcoholic	Alcoholic	Nonalcoholic	Alcoholic	Nonalcoholic
Serum						
GSA	0.085 ± 0.058	0.307 ± 0.222‡	0.086 ± 0.093	0.203 ± 0.094*	0.043 ± 0.030	0.390 ± 0.028§
Urea	3.53 ± 1.58	5.62 ± 2.21†	3.12 ± 1.49	4.52 ± 1.24	2.50 ± 1.31	6.92 ± 0.877‡
	(n = 22)	(n = 7)	(n = 16)	(n = 6)	(n = 11)	(n = 2)
Urine						
GSA	11.8 ± 8.30	29.8 ± 19.0†	8.19 ± 5.41	21.8 ± 7.82‡	4.46 ± 1.72	39.7 ± 19.6§
Urea	175 ± 58.4	231 ± 84.2	188 ± 102	216 ± 65.5	130 ± 61.6	231 ± 81.3
	(n = 21)	(n = 7)	(n = 17)	(n = 6)	(n = 11)	(n = 2)

NOTE. Results were compared by Student's *t*-test. *P* values indicate significant differences from alcoholic cirrhotic levels.

*$P < .05$.

†$P < .01$.

‡$P < .001$.

§$P < .0001$.

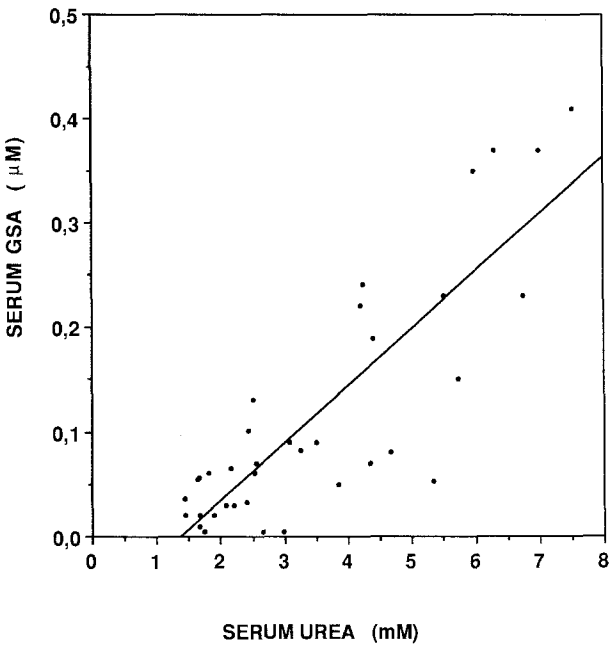

Fig 1. Relation between serum urea levels and corresponding serum GSA levels in Child-Turcotte B and C cirrhotic patients.

biosynthesis is not disturbed. However, another explanation for these normal levels could be that a normally functioning kidney in cirrhotic patients is itself capable of synthesizing enough arginine and homoarginine. Indeed, renal biosynthesis of arginine has long been recognized.[10,11]

The biosynthesis of guanidinoacetic acid primarily occurs in the kidney, whereas its methylation to creatine takes place mainly in the liver.[12] Our results clearly show that serum guanidinoacetic acid levels are increased with increased liver damage. This suggests that the biosynthesis of creatine is disrupted in the damaged liver. The increased retention of guanidinoacetic acid in blood could be a consequence of liver failure. In addition, it is noteworthy that in renal failure decreased levels of guanidinoacetic acid are found in serum.[13]

It has long been accepted that urea biosynthesis in cirrhotic patients is decreased. Serum and urinary excretion levels of GSA in our cirrhotic patients are clearly and significantly decreased as compared with control levels, which suggests a decreased biosynthesis. This could denote a metabolic relationship between urea and GSA. Moreover, Fig 1 clearly illustrates a positive linear correlation between serum urea and GSA levels. A significant positive linear correlation is also found in the Child-Turcotte C subgroup ($r = .847$, $P < .001$). The correlation coefficient in the total cirrhotic patient group (A, B, and C) is lower ($r = .608$, $P < .0001$). This could be explained by the fact that in the A subgroup some patients still have a normal residual urea production capacity, along with those with a subnormal or decreased capacity. Since there is a good correlation between serum urea levels and serum GSA levels in our cirrhotic patients, we will investigate whether GSA can be used as a parameter for actual, residual urea production capacity. Could GSA levels be used as a parameter or an indicator for liver dysfunction? Brewer et al[14] and Hansen and Poulsen[15] demonstrated that the urea production capacity or the daily urea biosynthesis rate is a parameter for the residual functional liver mass in rats. Müting et al[3] proposed the application of this parameter in man. As for the correlation between urea and GSA, GSA can perhaps be used as a complementary parameter for residual liver detoxification capacity.

Maier and Gerok[2] and Müting et al[3] showed that the daily urea nitrogen synthesis rate was decreased in cirrhotic patients. The results presented in Table 3 clearly show lower urea levels in the alcoholic cirrhotic group. Moreover, whereas in the total cirrhotic subgroups only B and C patients showed significantly lower serum urea levels as compared with control levels (Table 1), serum urea levels of alcoholic cirrhotic patients are all significantly lower than control urea levels (A, $P = .004$; B, $P = .0002$; and C, $P < .0001$). Alcoholic cirrhotic patients also have significantly lower GSA levels than nonalcoholic cirrhotic patients. The differences seem even more pronounced than those found for urea. These results clearly show that urea biosynthesis, and perhaps ammonia-nitrogen metabolism, is more disturbed and certainly more decreased in alcoholic cirrhotic patients than in nonalcoholic cirrhotic patients. As a consequence of decreased urea biosynthesis and the metabolic relationship between urea and GSA, we also found lower GSA levels in alcoholic cirrhotic patients than in nonalcoholic ones.

In conclusion, the metabolic relationship between urea and GSA demonstrated earlier in the pathobiochemistry of uremia and hyperargininemia is also seen in cirrhotic patients. The results of this study also show that the biosynthesis of urea and GSA is significantly lower in cirrhotic patients with an alcoholic origin than in cirrhotic patients with a nonalcoholic origin.

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