Pharmacokinetics of Guanidinosuccinic Acid in Rat Blood and Cerebrospinal Fluid

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Summary: Guanidinosuccinic acid (GSA), a uremic toxin, and its excess accumulation in the CSF under uremic conditions is thought to produce neural excitotoxicity. It is important to understand the manner of GSA distribution/elimination from the circulating blood and CSF and its alteration in the presence of renal failure. The purpose of this study was to evaluate the kinetics of GSA in the circulating blood using a rat model of cisplatin-induced renal failure and GSA transport between the circulating blood and CSF. The AUCinf and t1/2 of GSA in cisplatin-treated rats were approximately 7-fold greater than those in normal rats. The CLtot of GSA in cisplatin-treated rats was reduced by 88% compared with normal rats, whereas the Vss of GSA did not differ between normal and cisplatin-treated rats. These results suggest that the renal elimination of GSA is attenuated in cisplatin-treated rats. In normal rats, the elimination clearance of GSA from the CSF (15.5 µL/(min·rat)) was found to be 88-fold greater than its blood-to-CSF influx clearance (0.176 µL/(min·rat)). Thus, the greater elimination clearance of GSA from the CSF, compared with the influx clearance, may contribute to the maintenance of a low GSA concentration in the CSF.

Keywords: blood-cerebrospinal fluid barrier; cerebrospinal fluid; glomerular filtration; guanidinosuccinic acid; renal failure; uremic toxin

Introduction

Guanidinosuccinic acid (GSA), a uremic toxin, is a guanidino compound like guanidinoacetic acid (GAA) and creatinine (CTN).1 In the brain, GSA activates N-methyl-D-aspartate-type L-glutamate receptors and inhibits γ-aminobutyric acid receptors.2 Because intracerebroventricular administration of GSA induces convulsion in mice,3 it is considered that excess GSA accumulation in the brain/CSF will lead to a neural excitatory response and excitotoxicity.

In chronic renal failure, and thus uremia, serum GSA levels are increased because of the reduction in GSA clearance in the kidney and an increase in GSA synthesis.4,5 De Deyn et al. reported that the GSA concentration in the serum and cerebrospinal fluid (CSF) of patients with chronic renal insufficiency is raised to 58.0 µM and 31.8 µM, respectively.6 These values are 161-fold and 353-fold higher than those in the serum and CSF of control patients,5,6 implying that the increase in the GSA level in the plasma/serum causes an increase in the GSA concentration in the CSF. The GSA level in the brain is also increased in the relation to the concentration of renal insufficient markers.7 Because CSF is in direct contact with the cerebral interstitial fluid, the modulation of the GSA concentration in the CSF could offer a therapeutic strategy for treatment of a seizure in uremia induced by cerebral excess GSA. The exchange of compounds between the circulating blood and CSF is regulated by the blood-CSF barrier (BCSFB), which is formed by the tight-junctions of choroid plexus epithelial cells.8 Our previous studies have revealed that GAA and CTN are eliminated from the CSF via carrier-mediated transport processes at the BCSFB.9,10 Thus, it is possible that BCSFB-mediated transport plays a role in the control of the GSA concentration in the CSF.

Taking these findings into consideration, it is important to understand the manner of GSA distribution/elimination from the circulating blood and CSF and its alteration in renal failure. The purpose of this study was to evaluate the kinetics of GSA in the circulating blood in a rat model of renal failure produced by cisplatin administration. Moreover, GSA transport between the
circulating blood and CSF was examined by integration plot analysis and following intracerebroventricular administration.

**Materials and Methods**

**Animals:** Adult male Wistar rats (150–200 g) were purchased from Japan SLC (Hamamatsu, Japan). A rat model of cisplatin-induced renal failure (cisplatin-treated rats) was produced by intraperitoneal administration of 5 mg/kg cisplatin (Wako Pure Chemical Industries, Osaka, Japan) and was used 3 days after treatment.\(^9\) As the control, nothing was injected to the rats, i.e., normal rats. Plasma CTN concentrations in these rats were measured using a commercial kit (Creatinine-Test Wako; Wako Pure Chemical Industries). All experiments were approved by the Animal Care Committee, University of Toyama.

**GSA plasma pharmacokinetic studies:** GSA (Sigma-Aldrich, St. Louis, MO) was administered to normal and cisplatin-treated rats via the femoral vein (1 µmol/kg), and plasma was obtained. The total body clearance (CL\(_{\text{tot}}\)), elimination half-life (t\(_1/2\)), and distribution volume at steady state (V\(_{\text{ss}}\)) were calculated based on the area under the plasma concentration-time curve from time zero to infinity (AUC\(_{\text{inf}}\)), the mean residence time (MRT) and the elimination rate constant (l). Because the concentration of endogenous GSA in the plasma of cisplatin-treated rats was found to be 0.309 µM, the exogenous GSA concentration in the plasma of cisplatin-treated rats after intravenous administration was obtained by subtracting this value (0.309 µM) from the observed GSA concentrations in the plasma. The GSA concentrations in the plasma of normal rats remain to be observed because the endogenous GSA concentration in the plasma of normal rats was under the limit of quantification (<0.1 µM). The details are included in **Supplemental materials**.

**Determination of blood-to-CSF influx clearance of GSA:** The in vivo blood-to-CSF influx clearance of GSA was evaluated by measuring the GSA concentrations in rat plasma and CSF after intravenous administration (12.5 µmol/kg). The apparent blood-to-CSF influx clearance (CL\(_{\text{app,CSF}}\)) was determined as described previously.\(^9\) The details are included in **Supplemental materials**.

**In vivo GSA elimination from the CSF:** The in vivo GSA elimination from rat CSF was evaluated by measuring the GSA concentration in the CSF after intracerebroventricular administration (0.05 µmol/kg), and the distribution volume of the CSF (V\(_{d,\text{CSF}}\)), the elimination rate constant (k\(_e\)), and the apparent elimination clearance from the CSF (CL\(_{\text{app,CSF}}\)) were determined as described previously.\(^9\) The details are included in **Supplemental materials**.

**Quantification of GSA concentrations in samples:** The GSA concentrations in plasma (50 µL) and CSF (20 µL) were measured by high-performance liquid chromatography/tandem mass spectrometry using an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA) coupled to an API 4000\(^\text{TM}\) mass spectrometer (AB SCIEX, Foster City, CA). The details of the methods are included in **Supplemental materials**.

**Statistical analysis:** The kinetic parameters are represented as the mean ± SD. Other data are expressed as the mean ± SEM. The significance of differences was evaluated by an unpaired two-tailed Student’s t-test.

**Results and Discussion**

**GSA elimination from blood in normal and cisplatin-treated rats:** The CTN concentrations in the plasma of cisplatin-treated rats (0.919 ± 0.061 mg/dL) were 2-fold higher than those in normal rats (0.460 ± 0.033 mg/dL, p < 0.01). It is reported that a 2-fold increase of CTN concentration in serum/plasma is related to the decrease of creatinine clearance by approximately 80% and the impairment of renal proximal tubules.\(^11,12\) Thus, it is indicated that the cisplatin treatment of rats in this study induced the injury of renal proximal tubules, and thus the renal dysfunction. **Figure 1A** shows the time-profile of the GSA concentration in plasma of normal and cisplatin-treated rats after intravenous administration of 1 µmol/kg GSA. Although the plasma GSA concentration declined in a biexponential manner both in normal and cisplatin-treated rats, the GSA concentration in cisplatin-treated rats was at all the examined times significantly higher than that in normal rats. The kinetic parameters of GSA in these rats are summarized in **Table 1**. The AUC\(_{\text{inf}}\) and t\(_{1/2}\) of GSA in cisplatin-treated rats were 7.3-fold and 7.3-fold greater than in normal rats, respectively. The CL\(_{\text{tot}}\) of GSA in cisplatin-treated rats was reduced by 88% compared with normal rats, whereas the V\(_{\text{ss}}\) of GSA was not significantly different between normal and cisplatin-treated rats. These results suggest

![Fig. 1](https://example.com/fig1.png)

**Fig. 1.** (A) Time-profile of the plasma GSA concentration after intravenous administration of GSA in normal and cisplatin-treated rats; (B) the blood-to-CSF influx transport of GSA in normal rats; (C) GSA elimination from the CSF after intracerebroventricular administration of GSA in normal rats. (A) GSA (1 µmol/kg) was given intravenously to normal (open circles, n = 5) and cisplatin-treated (open squares, n = 3) rats. Each point represents the mean±SEM. (B) GSA (12.5 µmol/kg) was injected via the femoral vein into normal rats. The Y-axis shows the apparent CSF-to-plasma concentration ratio. The initial uptake of GSA by the CSF was evaluated by integration plot analysis using the following equation: K\(_{\text{app}}\)(t) = CL\(_{\text{app,CSF}}\) × AUC\(_{\text{inf}}\)/C\(_{\text{p}}\)(t) + Vi. Each point represents the mean±SEM (n = 3). (C) The GSA concentration in the CSF of normal rats was evaluated after intracerebroventricular administration of GSA (0.05 µmol/kg). The values are expressed as the percentage of the dose remaining per millilitre CSF. Each point represents the mean±SEM (n = 4).
analyze the CL_{inf,CSF}. The apparent CSF-to-plasma concentration to obtain a GSA concentration in the CSF high enough to The dose of GSA for this study was settled as 12.5 µmol/kg in intravenous administration of 12.5 µmol/kg GSA was performed in the GSA level in the CSF is correlated with that in the plasma. (as 37.9 « rats at 60 min after intravenous administration of 1 µmol/kg reported to injure renal proximal tubules,16) tubular secretion may be involved in the residual GSA clearance (35%) in the rat kidney. basolateral membrane of the renal proximal tubule cells in rats and revealed that GAA and CTN, which are guanidino compounds transported by OCT3/SLC22A3 and/or creatine transporter (CRT/SLC6A8) at the BCSFB.9,10) Although the contribution of these transporter 3 (OCT3/SLC22A3) and/or creatine transporter (CRT/SLC6A8) at the BCSFB.9,10) Although the contribution of these transporters to the elimination of GSA from the CSF will need to be assessed in further studies, these transporters could be candidates for the elimination of GSA from the CSF. In conclusion, our results indicate that GSA elimination from the circulating blood is attenuated in renal failure. Kinetic analysis implies that this attenuation is caused by the dysfunction of glomerular filtration and/or renal proximal tubule secretion. Moreover, the greater elimination clearance of GSA from the CSF than the blood-to-CSF influx clearance is thought to explain the low CSF-to-blood concentration ratio of GSA under normal and pathological conditions.

**References**


| Table 1. Pharmacokinetic parameters of GSA in normal and cisplatin-treated rats |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                  | Normal rats     | cisplatin-treated rats |
| ACU_{inf} (µmol-min/L)          | 273 ± 141       | 1,999 ± 641³     |
| t_{1/2} (min)                   | 42.1 ± 26.8     | 306 ± 116³       |
| CL_{inf} (µL/min-rat)           | 867 ± 343       | 107 ± 34³        |
| V_{ss} (mL/rat)                 | 40.1 ± 10.8     | 43.7 ± 8.8       |

Values are represented as the mean ± SD (n = 5, normal rats; n = 3, cisplatin-treated rats). *p < 0.01, significantly different from normal rats.

that the elimination of GSA is attenuated in cisplatin-treated rats. It has been reported that intraperitoneal cisplatin administration induces hardly any hepatic dysfunction because the concentrations of biomarkers for hepatic injury in the plasma of rats treated with cisplatin (5.0 mg/kg, i.p.) were not significantly altered.¹³ Moreover, it has been reported that GSA is hardly metabolized in the liver or circulating blood.¹⁴ Taking these points into consideration, it is indicated that the metabolism of GSA in the liver and circulating blood did not contribute to the alteration of plasma concentration of GSA in cisplatin-treated rats. Consequently, urinary excretion is suggested to be the major pathway of GSA elimination from the circulating blood in rats.

In normal rats, the glomerular filtration rate (GFR) has been reported to be 550–610 µL/(min-rat).¹⁵ The CL_{tot} of GSA in normal rats was 1.4–1.6 times greater than this GFR value (Table 1), indicating that glomerular filtration is responsible for approximately 65% of GSA clearance in the kidney. Because cisplatin administration reduced the GSA clearance by 88% (Table 1) and is reported to injure renal proximal tubules,¹⁰ tubular secretion may be involved in the residual GSA clearance (35%) in the rat kidney. This secretion process of GSA involves transporters expressed in renal proximal tubule epithelial cells. Toyohara et al. reported that the plasma concentration of GSA in five-sixths nephrectomy (Nx) rats renal-specifically harboring human organic anion transporting polypeptide 4C1 (OATP4C1/SLC04C1), which is localized at the basolateral membrane of the renal proximal tubule cells in rats and humans,¹⁷ was reduced compared with that in wild-type Nx rats.¹⁵ There is a possibility that OATP4C1 contributes to this residual GSA clearance in the rat kidney.

**GSA transport between the circulating blood and CSF:** At 60 min after intravenous administration of 1 µmol/kg GSA, the GSA concentration in the CSF of cisplatin-treated rats (0.134 ± 0.038 µM, n = 3) is significantly higher (2.9-fold, p < 0.01) than that of normal rats (0.0457 ± 0.0010 µM, n = 5). Because the GSA concentration in the plasma of normal and cisplatin-treated rats at 60 min after intravenous administration of 1 µmol/kg GSA was determined to be 1.32 ± 0.23 µM and 3.75 ± 0.21 µM, respectively (Fig. 1A), the apparent CSF-to-plasma concentration ratio of GSA in normal and cisplatin-treated rats was obtained as 37.9 ± 5.9 µM/mL CSF (n = 5) and 36.7 ± 8.5 µM/mL CSF (n = 3), respectively. There is no significant difference between the apparent CSF-to-plasma concentration in normal and cisplatin-treated rats (p > 0.05). These results suggest that the elevation of the GSA level in the CSF is correlated with that in the plasma. Furthermore, an integration plot analysis for normal rats after intravenous administration of 12.5 µmol/kg GSA was performed in order to determine the in vivo blood-to-CSF transport clearance. The dose of GSA for this study was settled as 12.5 µmol/kg in order to obtain a GSA concentration in the CSF high enough to analyze the CL_{inf,CSF}. The apparent CSF-to-plasma concentration ratio of normal rats was time-dependently increased until 5 min after intravenous GSA administration (Fig. 1B). The CL_{inf,CSF} for GSA was found to be 0.702 ± 0.141 µL/(min-mL CSF). Since the CSF volume in rat cerebroventricles is assumed to be 250 µL,¹⁸ the CL_{inf,CSF} was calculated as 0.176 ± 0.035 µL/(min-rat). To compare this value with the elimination clearance of GSA from the CSF, we examined the time-profile of the GSA concentration in the CSF of normal rats after intracerebroventricular administration of GSA. Because the residual GSA concentration in the CSF at 60 min after administration was not significantly altered compared with that at 30 min after administration (p > 0.05), it is indicated that the decrease of residual GSA concentration in the CSF up to 30 min reflects the elimination of GSA from the CSF. The residual GSA concentration in the CSF exhibited a time-dependent decrease with a half-life of 13.6 ± 2.9 min (Fig. 1C). Since the k_d and V_d,CSF of GSA were found to be 0.0511 ± 0.0107 min⁻¹ and 303 ± 60 µL/rat, respectively, the CL_{inf,CSF} was calculated at 15.5 ± 4.5 µL/(min-rat). This CL_{inf,CSF} value was 88-fold greater than the CL_{inf,CSF} of GSA. De Deyn et al. reported that the GSA concentration in the CSF of both control patients (0.09 µM) and non-dialyzed patients with renal insufficiency (0.22–31.8 µM) is lower than that in serum (control patients, 0.36 µM; non-dialyzed patients with renal insufficiency, 0.87–58.0 µM).¹⁶ Our findings suggest that GSA is asymmetrically transported between the circulating blood and CSF, and this transport of GSA, including BCSFB-mediated processes, plays a role in the lower concentration of GSA in the CSF compared with the serum. Because the paracellular transport is restricted at the BCSFB, it is thought that some transporters are involved in the transcellular transport of GSA at the BCSFB. It has been reported that some solute carrier (SLC) family members contribute to the transport of guanidino compounds including GSA. Toyohara et al. have reported that OATP4C1 in the kidney is involved in the GSA elimination from the circulating blood.¹⁵ In addition, we have revealed that GAA and CTN, which are guanidino compounds as well as GSA, were eliminated from the CSF via organic cation transporter 3 (OCT3/SLC22A3) and/or creatine transporter (CRT/SLC6A8) at the BCSFB. Although the contribution of these transporters to the elimination of GSA from the CSF will need to be assessed in further studies, these transporters could be candidates for the elimination of GSA from the CSF.

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