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Note

Pharmacokinetics of Guanidinosuccinic Acid in Rat Blood and Cerebrospinal Fluid

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Summary: Guanidinosuccinic acid (GSA) is 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 AUC_{inf} and $t_{1/2}$ of GSA in cisplatin-treated rats were approximately 7-fold greater than those in normal rats. The CL_{tot} of GSA in cisplatin-treated rats was reduced by 88% compared with normal rats, whereas the V_{ss} 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

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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.[10] 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_{tot}), elimination half-life ($t_{1/2}$), and distribution volume at steady state (V_{ss}) were calculated based on the area under the plasma concentration-time curve from time zero to infinity (AUC_{inf}), the mean residence time (MRT) and the elimination rate constant (λ). 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 ($CL_{inf,CSF}$) 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 ($V_{d,CSF}$), the elimination rate constant (k_{el}), and the apparent elimination clearance from the CSF ($CL_{eff,CSF}$) 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™ 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_{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_{tot} of GSA in cisplatin-treated rats was reduced by 88% compared with normal rats, whereas the V_{ss} of GSA was not significantly different between normal and cisplatin-treated rats. These results suggest

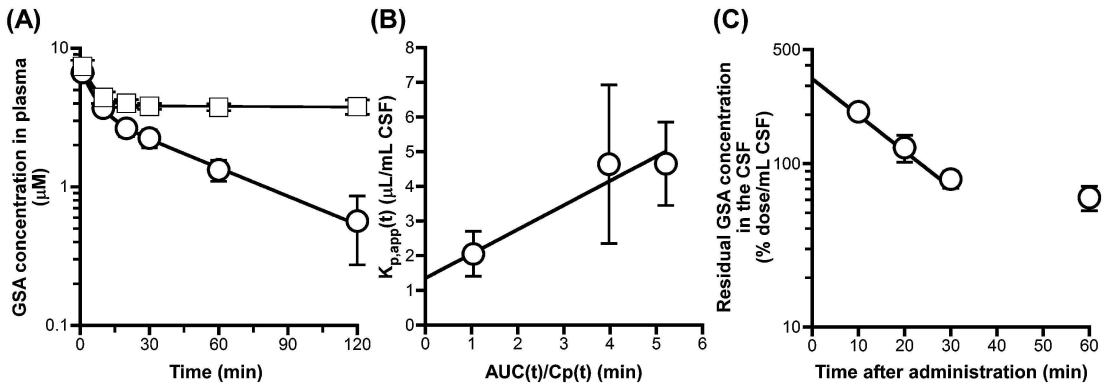

Fig. 1. (A) Time-profile of the plasma GSA concentration after intravenous administration of GSA in normal and cisplatin-treated rats; (B) the blood-to-CSF influx transport of GSA in normal rats; (C) GSA elimination from the CSF after intracerebroventricular administration of GSA in normal rats
(A) GSA (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: $K_{p,app}(t) = CL_{inf,CSF} \times AUC(t)/C_p(t) + Vi$. 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 milliliter CSF. Each point represents the mean ± SEM ($n = 4$).