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Comparison of busulfan pharmacokinetics between four-times-daily and once-daily administration in pediatric patients: a preliminary prospective observational trial

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32

Abstract

Therapeutic drug monitoring (TDM) of busulfan (BU) is useful for achieving the target area under the curve (AUC) because its effective plasma concentration range is narrow. This preliminary observational study evaluated the pharmacokinetic (PK) parameters of BU administered four times daily or once daily to pediatric patients. The plasma concentrations were measured at both the test dose and first dose, and the doses on day 1 and days 2–4 were determined based on each TDM. A comparison of PK parameters between four-times-daily and once-daily administration was performed for both the test dose and first dose of BU. Of the 11 patients, five received four-times-daily BU and six received once-daily BU. The V_d for once-daily administration was higher than that for four-times-daily administration for the first dose but not for the test dose. The ratio of actual AUC for the first dose to estimated AUC guided by the test dose was lower with once-daily administration than with four-times-daily administration. These results indicate that the PK parameters of BU administered once daily are challenging to predict based on the TDM of the test dose. TDM should be considered on day 1 to achieve the target AUC, especially with once-daily administration.

Keywords: busulfan; hematopoietic stem cell transplantation; test dose; therapeutic drug

51 monitoring; pharmacokinetics

Introduction

Busulfan (BU) is an alkylating agent that is often used in hematopoietic stem cell transplantation (HSCT). A higher area under the curve (AUC) of BU is associated with toxicity, such as sinusoidal obstruction syndrome (SOS), and a lower AUC results in graft failure, relapse, and graft-versus-host disease (GVHD) [1–3]. The safety and efficacy of the intravenous four-times-daily administration of BU was confirmed in a phase II trial [4]. BU once-daily method has also been found to be well-tolerated, and a comparative study between four-times-daily and once-daily administration of BU in adolescents and adults was conducted [5,6]. The study indicated that the toxicity and pharmacokinetics (PK) of BU were similar between the two methods. However, a BU PK comparison study between four-times-daily and once-daily administration methods in pediatric population has not been reported. Because BU has a large inter-individual variability and a narrow effective range, therapeutic drug monitoring (TDM) is useful to maximize the efficacy and minimize the toxicity of BU. The PK of BU varies among patients, and therefore, determination of PK parameters of BU using the test dose before conditioning chemotherapy may be useful to predict BU exposure [7–9]. However, its effectiveness/usefulness in pediatric patients is not well established. In this study, we aimed to compare PK after once-daily and four-times-daily administration of BU in

pediatric patients and the usefulness of the test dose prediction of BU PK. We analyzed PK parameters, including the elimination rate constant (k_{el}), clearance (CL), distribution volume (Vd), and AUC. k_{el} represents the fraction of drug eliminated from the body over a certain period. The CL is the plasma volume of a drug eliminated in a given period, indicating its ability to eliminate it. Vd is the total amount of drug in the body divided by its plasma concentration, relating to the disposition of drug to tissues and organs. The AUC is the integral value of the plasma drug concentration-time curve that reflects the total drug exposure. We compared these parameters between four-times-daily and once-daily administration for the test and the first dose. To verify the usefulness of the test administration, we also calculated the coincident ratios of the actual AUC for the first dose and the estimated AUC for the test dose ($actAUC/estAUC$) using the four-times-daily and once-daily dosing regimens. This ratio provides a measure of the predictive performance of the AUC based on test dose analysis.

Methods

Patients

Patients who received intravenous BU and underwent plasma concentration measurements between April 2021 and December 2023 at Hokkaido University Hospital were prospectively evaluated. The present study was approved by the Ethical Review Board for Life Science and Medical Research of Hokkaido University Hospital (approval number: 022-0319) and was conducted in accordance with the Declaration of Helsinki and STROBE statement. Informed consent was obtained from each participant or proxy.

Treatment regimens and methods of determining plasma concentration, PK, and AUC of BU

The conditioning treatment included intravenous BU for four days, and the test dose was implemented appropriately one week before the conditioning treatment, as in a previous study [9]. The test dose was 0.6 mg/kg in all patients. BU TDM was performed at the test dose and first dose of the conditioning treatment. The BU doses on day 1 and days 2–4 were determined based on TDM of the test dose and first dose, respectively. The target AUC of BU was established in a range of 65–75 mg*h/L by the physician according to individual disease, and BU exposure was expressed in mg*h/L by consensus statement

[10]. BU was administered over two hours in four-times-daily and over three hours in once-daily method. Plasma samples were collected at 5 min, 1, 2, 4, and 8 h after the termination of BU administration of the test dose and first dose of once-daily method, and at 5 min, 1, 2, and 4 h before the next administration of the first dose of four-times-daily method. Urine concentration was measured in one patient, who provided informed consent, until 13 h after BU initiation. Plasma and urine concentrations of BU were measured using liquid chromatography-tandem mass spectrometry (LC/MS/MS). The actual AUC values were calculated using the trapezoidal method. According to TDM, the estimated AUC of the test dose was estimated using one-compartment model. The PK parameters, such as k_{el} , CL, and Vd, were calculated using one-compartment model. GVHD was assessed according to the Mount Sinai Acute GVHD International Consortium [11]. Hepatic SOS and veno-occlusive disease were assessed according to the European Society for Blood and Marrow Transplantation classification [12].

Supportive care

Seizures were prevented using levetiracetam (20 mg/kg/day) from the day before BU administration until the day after the completion of BU treatment. SOS was prevented using ursodeoxycholic acid (10 mg/kg/day) and dalteparin (75 U/kg/day) before the

conditioning therapy until day 30 after transplantation. In case of occurrence of SOS,
defibrotide (6.25 mg/kg every 6 h) was used as treatment option.

Outcomes

The primary endpoint of this study was the comparison of PK parameters of BU
between four-times-daily and once-daily administration methods. The secondary
endpoints of the study were the coincident ratios of the (actAUC/estAUC) in four-times-
daily and once-daily methods. Treatment efficacy and toxicity were also evaluated.

Statistical analysis

Early clinical events during 100 days after transplantation were visually
described. Differences in PK parameters between four-times-daily and once-daily
methods were assessed using the Mann–Whitney *U* test. The ratio of the actual AUC for
the first dose and the estimated AUC for the test dose and that of Vd for the first dose and
test dose were assessed using Pearson’s correlation test. All statistical analyses were
performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama,
Japan), which is a graphical user interface for R (The R Foundation for Statistical
Computing, Vienna, Austria). Differences were considered statistically significant at $p <$

137 0.05.

Results

Patient characteristics

The patient characteristics are presented in Table 1 and Supplemental Table S1. Our cohort consisted of 11 patients with a median age at HSCT of 7.6 years old (range: 0.4–11.8). The underlying diseases were as follows: malignant disease in two patients, inborn errors of immunity in seven patients, and inborn errors of metabolism in two patients. The conditioning regimen and target AUC of BU (mg*h/L) were as follows: BU (65)/cyclophosphamide (CY) and BU (72)/melphalan (Mel) in two patients each, and fludarabine (Flu)/BU (65) regimen in seven patients. The BU doses on day 1 were determined according to TDM of the test dose for all patients. After BU TDM of the first dose on day1, its doses on days 2–4 were adjusted as necessary.

Treatment efficacy and toxicity associated with BU between four-times daily and once-daily methods

The clinical outcomes are summarized in Table 2 and Fig. 1. Of the 11 patients treated with BU conditioning therapy, five received four-times-daily administration and six received once-daily administration. Because the sample size was small, we could not evaluate the differences in efficacy and toxicity between BU administration methods. The

median number of neutrophil engraftment days was 15 (range, 11–18) in four-times-daily and 13.5 (11–22) in once-daily method. No graft failure was observed in any patient. SOS developed in one and three patients, and very severe SOS developed in one patient each in four-times-daily and once-daily methods, respectively. Although most patients with allogeneic HSCT developed acute GVHD (aGVHD), the severity was of grade I or II, and the disease was controllable.

Comparison of PK parameters for the test dose and first dose of BU between four-times daily and once-daily administration methods

The PK parameters for the test dose and first dose and for both methods are described in Table 3. AUC, k_{el} , CL, and Vd for the test dose (0.6 mg/kg/dose) were not significantly different between four-times-daily and once-daily methods. Similar to the test dose, k_{el} and CL were not significantly different between four-times-daily and once-daily methods for the first dose PK analysis. In contrast, Vd in once-daily method was higher than that in four-times-daily method for the first dose PK analysis (1.03 ± 0.21 L/kg in four-times-daily method vs. 1.71 ± 0.54 L/kg in once-daily method, $p = 0.02$). Consistently, cumulative AUC calculated for the first dose PK analysis was lower in once-daily method than that in four-times-daily method (63.20 ± 8.68 mg*h/L in four-times-

daily method vs. 38.30 ± 7.70 mg*h/L in once-daily method, $p < 0.01$). In addition, the coincident ratio of actAUC/estAUC was also lower in once-daily method than that in four-times-daily method ($88.60 \pm 5.73\%$ in four-times-daily method vs. $58.5 \pm 5.24\%$ in once-daily method, $p < 0.01$).

The difference in actAUC/estAUC between four-times-daily and once-daily methods indicated intra-individual factors associated with the test dose and first dose. Conditions, such as administration time, hydration, and concomitant medication, were the same in the test dose and first dose. Therefore, we considered that the dose differences between the test dose and first dose or between four-times-daily and once-daily methods influenced Vd and actAUC/estAUC. Consequently, we evaluated the correlation between the dose ratio for the test dose and first dose and that for Vd or actAUC/estAUC (Fig. 2). The Vd ratio correlated positively with the dose ratio for the test dose and first dose ($r = 0.604$, $p = 0.049$, Fig. 2a). In contrast, actAUC/estAUC showed negative correlation with the dose ratio for the test dose and first dose ($r = -0.79$, $p < 0.01$, Fig. 2b).

Trend in BU plasma concentration and cumulative amount of BU excreted in urine

Although no significant difference was observed in CL between four-times-daily and once-daily methods in the first dose PK analysis (4.23 ± 1.74 mL/min/kg in four-

192 times-daily method vs. 5.50 ± 2.22 mL/min/kg in once-daily method, $p = 0.33$), CL in
193 once-daily method tended to be higher than that in four-times-daily. Therefore, we
194 determined BU urinary excretion in one patient. BU urinary concentration for the test
195 dose was below the detection sensitivity (less than 25 ng/mL) and could not be detected
196 because of the low dosage; however, it was detected for the first dose (3.4 mg/kg) (Fig.
197 3), and BU urinary excretion ratio was calculated as 2.46% during approximately the first
198 12 h after BU administration.

Discussion

In this study, we evaluated the PK parameters of four-times-daily and once-daily methods (Table 3). Previous comparative studies in adolescents and adults indicated that PK parameters, such as Vd, CL, and cumulative AUC, did not differ between four-times-daily and once-daily methods [5,6]. Similar to the adult data, AUC, k_{el} , CL, and Vd did not differ between the two methods for the test dose. The age and weight distributions between the two methods were not balanced, and patients in the four-times-daily group tended to be younger and lighter (Supplemental Table S1). These patients generally had higher CL for BU and required higher doses. In addition, two infant patients whose Vd tended to be higher were included in the four-times-daily group [13]. Although the number of patients was small and analysis by age or weight was not available, these factors could have affected the PK differences in the first dose TDM. However, there was no difference in PK parameters between the two groups at the test dose (fixed at 0.6 mg/kg); we considered that interindividual variability in PK characteristics was similar between the two groups. The study used the same conditions except for the administration time (two vs. three hours) in the TDM of the test dose; administration time did not affect PK parameters. A previous study that simulated various doses and administration times showed similar AUC but did not show higher C_{max} in the once-daily dose and three-hour

administration time [14], consistent with our AUC results for the test dose. The k_{el} and CL values did not differ significantly at the first dose between the four-times-daily and once-daily methods. Although the CL value in once-daily method varied in pediatric patients from 1.47 to 6.90 mL/min/kg according to previous reports [15–17], it was estimated to be 5.50 ± 2.22 mL/min/kg in this study. In contrast, cumulative AUC and actAUC/estAUC ratio in once-daily method were significantly lower than those in four-times-daily method for the first dose. In addition, Vd in once-daily method was higher than that in four-times-daily method, suggesting that an increase in Vd induced a decrease in BU plasma concentration and AUC. This study suggests that BU dose adjustment according to the TDM of the test dose may be insufficient to achieve the target AUC in the once-daily method because of the differences in Vd between the two methods.

In our correlation analysis, actAUC/estAUC ratio was strongly correlated with the dose ratio for both the test dose and first dose (Fig. 2b). In contrast, Vd ratio exhibited a significant negative correlation with the dose ratio (Fig. 2a). Consequently, we considered that dose differences between the test dose and first dose affected Vd and AUC. In other words, an increase in the first dose in once-daily methods induced an increase in Vd. We observed that the Vd in the once-daily method was higher than in the four-times-daily method, resulting in differences in actAUC/estAUC. These observations could have

235 been affected by dose differences. One example of PK alteration with dose differences is
236 the saturation of metabolic enzymes such as salicylic acid and phenytoin [18]. In such
237 cases, the drug CL was reduced; however, no significant effect on CL was observed in
238 this study. In by passive diffusion, Vd is specified by the transferability between the blood
239 and tissue and the rate of protein binding in the blood and tissue. For example, phenytoin
240 has a large Vd and a high protein-binding rate, and its Vd is known to increase when
241 plasma proteins are decreased or saturated [18]. Although BU has a large Vd and high
242 tissue transferability, its protein binding rate was reported to be less than 10% [19],
243 leading to a small effect of protein binding on Vd. However, it has been reported that
244 32.4% of BU binds irreversibly to plasma proteins by covalent binding through
245 nonspecific alkylation [20]. Considering the PK characteristics of BU, it is possible that
246 the high concentration of the once-daily dosing regimen saturates irreversible binding in
247 plasma proteins, resulting in enhanced BU transfer to tissues and increased Vd. Although
248 the mechanism inducing the Vd increase in pediatrics was not revealed, the tissue
249 transitional potential of BU and body composition differences between pediatrics and
250 adults [21] could be also the reasons for Vd change by BU dose. The present study
251 analyzed and compared the PK parameters of BU between four-times-daily method and
252 once-daily method in pediatric patients. Further studies comparing Vd between the four-

times-daily and once-daily methods are required.

The CL value of BU in once-daily method tended to be higher than that in four-times-daily method for the first dose although the difference was not significant. CL increases at high concentrations, for example, some small molecules, such as glucose, pass glomerular filtration and almost reabsorbed in the proximal tubule via transporters. They are reabsorbed to a limited extent because of the saturation of transport activity, and the urinary excretion rate increases [22]. Therefore, we speculated that BU urinary excretion increased, which was measured to be 2.46%, with high dose BU and plasma BU concentration (once-daily method) based on the mechanism described above (Fig. 3). However, considering that unchanged BU in the urine was 2% of the dose (orally 1 mg/kg every 6 h) reported by Hassan et al. [23], the urinary excretion in once-daily method did not increase.

To perform TDM, it is important to consider whether the test dose adjustment is useful. In our patients, dose adjustment for the test dose achieved the target AUC in four-times-daily, but not in once-daily method (Table 3). The higher degree of dose alteration between the first dose and test dose may have induced a difference in the target AUC in once-daily method (Fig. 2). Some studies have demonstrated no difference in CL values for the test dose and first dose in once-daily method [15,17,24]. However, a deviation

271 from AUC of more than 10% was confirmed in 29.0% patients [17] and 20% patients did
272 not achieve the target AUC after adjusting BU dose to the test dose [24]. Similarly, a case
273 report indicated the predicted AUC from the test dose deviated more than 10% in four of
274 five children [25]. Guidelines recommend the first dose of TDM, however, a test dose
275 strategy is not currently recommended [7,8]. Our data also support these guidelines,
276 particularly for once-daily method. In contrast, TDM on day 1 was performed in several
277 studies for both children and adults [3,16,26–30] and adjusting BU dose to achieve the
278 target AUC was good for improved clinical outcomes [28], including low incidence of
279 SOS [30] or acute GVHD [3] and superior efficacy [27]. In our patients, dose adjustment
280 according to TDM for the first dose showed that most treatment-related toxicities were
281 controllable, and graft failure was not observed (Fig. 1). The incidence of SOS was
282 approximately 20% in normal patients and up to 60% in high-risk patients [12]. The
283 incidence rate, except for two patients developing SOS with high-risk (one patient had
284 neuroblastoma and the other was infant), was similar to the previous report [12] (22.2%,
285 two of nine patients). Although two patients with high-risk SOS developed severe SOS,
286 they were treated with defibrotide and recovered. This study included two infants with a
287 higher SOS risk [9,13,31]. One reason is the high CL and Vd values in infants [31].
288 Considering these PK differences, TDM in infants might be more effective in reducing

289 the incidence of SOS. Although the number of participants in this study was small, we
290 believe that TDM can be effective in improving treatment outcomes, including BU
291 conditioning.

292 This study has some limitations. First, the number of patients was too small for
293 detailed analysis. Second, age, weight, disease, conditioning regimen, and target AUC of
294 BU were not uniform between the two groups. Third, genetic polymorphisms associated
295 with BU metabolism, which could influence the PK and AUC of BU, were not evaluated.
296 Finally, we did not examine the AUC after dose adjustment during the first TDM. These
297 data require further review to determine the association between AUC and clinical
298 outcomes. Consequently, the results of this study are preliminary, and further studies
299 evaluating the PK after four-times-daily and once-daily BU administration are warranted.

Conclusion

This preliminary observational study demonstrated that the V_d values of BU were different between four-times-daily and once-daily methods, resulting in underestimation of AUC guided by the test dose PK analysis, because the dose variation between the test and first BU administration was large in the two methods. Ideally, BU TDM and dose adjustment on day 1 would be better for achieving the target AUC.

Declaration

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Conflicts of interest

Atsushi Manabe is an associate Editor of International Journal of Hematology. The other authors declare that they have no conflict of interest.

Data availability

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

318

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322

323 **Authors' contributions**

324 AY, SH, and YT designed the study. AY, SH, and YT conducted the study and analyzed
325 the data. KN and KK measured BU plasma concentration. AY, SH, KN, KK, YT, YC, AM,
326 MS, and YT contributed to the writing of the manuscript. All authors reviewed the results
327 and approved the final version of the manuscript.

328

329 **Ethical approval**

330 All procedures performed in this study involving human participants were carried out in
331 accordance with the ethical standards of the institutional and/or national research
332 committee and the 1964 Helsinki Declaration and its later amendments or comparable
333 ethical standards. For this type of study, formal consent was waived by the Ethical Review
334 Board for Life Science and Medical Research at Hokkaido University Hospital.

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References

1. Bartelink IH, Lalmohamed A, van Reij EML, Dvorak CC, Savic RM, Zwaveling J, et al. Association of busulfan exposure with survival and toxicity after haemopoietic cell transplantation in children and young adults: a multicentre, retrospective cohort analysis. *Lancet Haematol*. 2016;3:e526–36.
2. Philippe M, Neely M, Rushing T, Bertrand Y, Bleyzac N, Goutelle S. Maximal concentration of intravenous busulfan as a determinant of veno-occlusive disease: a pharmacokinetic-pharmacodynamic analysis in 293 hematopoietic stem cell transplanted children. *Bone Marrow Transplant*. 2019;54:448–57.
3. Versluys AB, Boelens JJ, Pronk C, Lankester A, Bordon V, Buechner J, et al. Hematopoietic cell transplant in pediatric acute myeloid leukemia after similar upfront therapy: a comparison of conditioning regimens. *Bone Marrow Transplant*. 2021;56:1426–32.
4. Andersson BS, Kashyap A, Gian V, Wingard JR, Fernandez H, Cagnoni PJ, et al. Conditioning therapy with intravenous busulfan and cyclophosphamide (IV BuCy2) for hematologic malignancies prior to allogeneic stem cell transplantation: A phase II study. *Biol Blood Marrow Transplant*. 2002;8:145–54.
5. Ryu S-G, Lee J-H, Choi S-J, Lee J-H, Lee Y-S, Seol M, et al. Randomized comparison of four-times-daily versus once-daily intravenous busulfan in conditioning therapy for hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2007;13:1095–105.
6. Madden T, de Lima M, Thapar N, Nguyen J, Roberson S, Couriel D, et al. Pharmacokinetics of once-daily IV busulfan as part of pretransplantation preparative regimens: a comparison with an every 6-hour dosing schedule. *Biol Blood Marrow Transplant*. 2007;13:56–64.
7. Palmer J, McCune JS, Perales M-A, Marks D, Bubalo J, Mohty M, et al. Personalizing busulfan-based conditioning: considerations from the American Society for Blood and Marrow Transplantation Practice Guidelines Committee. *Biol Blood Marrow Transplant*. 2016;22:1915–25.
8. Zao JH, Schechter T, Liu WJ, Gerges S, Gassas A, Egeler RM, et al. Performance of busulfan dosing guidelines for pediatric hematopoietic stem cell transplant conditioning. *Biol Blood Marrow Transplant*. 2015;21:1471–8.
9. Sakashita K, Yoshida N, Muramatsu H, Ohtsuka Y, Watanabe K, Yabe M, et al. Allogeneic hematopoietic cell transplantation for juvenile myelomonocytic leukemia with a busulfan, fludarabine, and melphalan regimen: JPLSG JMML-11. *Transplant Cell Ther*. 2024;30:105.e1-105.e10.

10. McCune JS, Quinones CM, Ritchie J, Carpenter PA, van Maarseveen E, Yeh RF, et al. Harmonization of busulfan plasma exposure unit (BPEU): a community-initiated consensus statement. *Biol Blood Marrow Transplant*. 2019;25:1890–7.
11. Harris AC, Young R, Devine S, Hogan WJ, Ayuk F, Bunworasate U, et al. International, multicenter standardization of acute graft-versus-host disease clinical data collection: a report from the Mount Sinai acute GVHD international consortium. *Biol Blood Marrow Transplant*. 2016;22:4–10.
12. Corbacioglu S, Carreras E, Ansari M, Balduzzi A, Cesaro S, Dalle J-H, et al. Diagnosis and severity criteria for sinusoidal obstruction syndrome/veno-occlusive disease in pediatric patients: a new classification from the European society for blood and marrow transplantation. *Bone Marrow Transplant*. 2018;53:138–45.
13. Schechter T, Finkelstein Y, Doyle J, Verjee Z, Moretti M, Koren G, et al. Pharmacokinetic Disposition and Clinical Outcomes in Infants and Children Receiving Intravenous Busulfan for Allogeneic Hematopoietic Stem Cell Transplantation. *Biology of Blood and Marrow Transplantation*. 2007;13:307–14.
14. Kim Y, Moon S, Rhee SJ. Optimal Once-Daily Busulfan Administration in Pediatric Patients: A Simulation-Based Investigation of Intravenous Infusion Times. *Drug Des Devel Ther*. 2024;18:871–9.
15. Tse WT, Duerst R, Schneiderman J, Chaudhury S, Jacobsohn D, Kletzel M. Age-dependent pharmacokinetic profile of single daily dose i.v. busulfan in children undergoing reduced-intensity conditioning stem cell transplant. *Bone Marrow Transplant*. 2009;44:145–56.
16. Ohwada C, Yamazaki S, Shono K, Kayamori K, Hino Y, Oshima-Hasegawa N, et al. Pharmacokinetically guided, once-daily intravenous busulfan in combination with fludarabine for elderly AML/MDS patients as a conditioning regimen for allogeneic stem cell transplantation. *Int J Hematol*. 2021;114:664–73.
17. Ward J, Kletzel M, Duerst R, Fuleihan R, Chaudhury S, Schneiderman J, et al. Single daily busulfan dosing for infants with nonmalignant diseases undergoing reduced-intensity conditioning for allogeneic hematopoietic progenitor cell transplantation. *Biol Blood Marrow Transplant*. 2015;21:1612–21.
18. Ludden TM. Nonlinear pharmacokinetics: clinical Implications. *Clin Pharmacokinet*. 1991;20:429–46.
19. Hassan M, Ehrsson H, Ehrnebo M, Wallin I, Smedmyr B, T6ttermann T, et al. Pharmacokinetic and metabolic studies of high-dose busulphan in adults*. *Eur J Clin Pharmacol*. 1989.
20. Ehrsson H, Hassan M. Binding of busulfan to plasma proteins and blood cells.

Journal of Pharmacy and Pharmacology. 1984;36:694–6.

21. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental pharmacology — drug disposition, action, and therapy in infants and children. *N Engl J Med*. 2003;349:1157–67.
22. Ghezzi C, Loo DDF, Wright EM. Physiology of renal glucose handling via SGLT1, SGLT2 and GLUT2. *Diabetologia*. Springer Verlag; 2018. p. 2087–97.
23. Hassan M, Ehrsson H, Ehrnebo M, Wallin I, Smedmyr B, Tötterman T, et al. Pharmacokinetic and metabolic studies of high-dose busulphan in adults. *Eur J Clin Pharmacol*. 1989;36:525–30.
24. Kletzel M, Jacobsohn D, Duerst R. Pharmacokinetics of a test dose of Intravenous busulfan guide dose modifications to achieve an optimal area under the curve of a single daily dose of intravenous busulfan in children undergoing a reduced-intensity conditioning regimen with hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2006;12:472–9.
25. Brooks KM, Jarosinski P, Hughes T, Kang E, Shah NN, Gall JB Le, et al. Test dose pharmacokinetics in pediatric patients receiving once-daily IV busulfan conditioning for hematopoietic stem cell transplant: a reliable approach? *J Clin Pharmacol*. 2018;58:332–9.
26. Andersson BS, Thall PF, Valdez BC, Milton DR, Al-Atrash G, Chen J, et al. Fludarabine with pharmacokinetically guided IV busulfan is superior to fixed-dose delivery in pretransplant conditioning of AML/MDS patients. *Bone Marrow Transplant*. 2017;52:580–7.
27. Bognàr TT, Kingma JSJ, Smeijsters EHE, van der Elst KCMK, de Kanter CTMK, Lindemans CAC, et al. Busulfan target exposure attainment in children undergoing allogeneic hematopoietic cell transplantation: a single day versus a multiday therapeutic drug monitoring regimen. *Bone Marrow Transplant*. 2023;58:762–8.
28. Philippe M, Goutelle S, Guitton J, Fonrose X, Bergeron C, Girard P, et al. Should busulfan therapeutic range be narrowed in pediatrics? Experience from a large cohort of hematopoietic stem cell transplant children. *Bone Marrow Transplant*. 2016;51:72–8.
29. Shao D-F, Li J-H, Hu T, Zhang Z-X, Zhang L, Li J-J, et al. Clinical outcomes of individualized busulfan-dosing in hematopoietic stem cell transplantation in Chinese children undergoing with therapeutic drug monitoring. *Bone Marrow Transplant*. 2022;57:473–8.
30. Gurlek Gokcebay D, Arman Bilir O, Şahin S, Ok Bozkaya İ, Ozbek NY. Role of therapeutic drug monitoring of intravenous Busulfan for prevention of sinusoidal obstructive syndrome in children. *Pediatr Transplant*. 2022;26:e14266.

31. Corbacioglu S, Hönig M, Lahr G, Stöhr S, Berry G, Friedrich W, et al. Stem cell transplantation in children with infantile osteopetrosis is associated with a high incidence of VOD, which could be prevented with defibrotide. Bone Marrow Transplant. 2006;38:547–53.

Figure legends

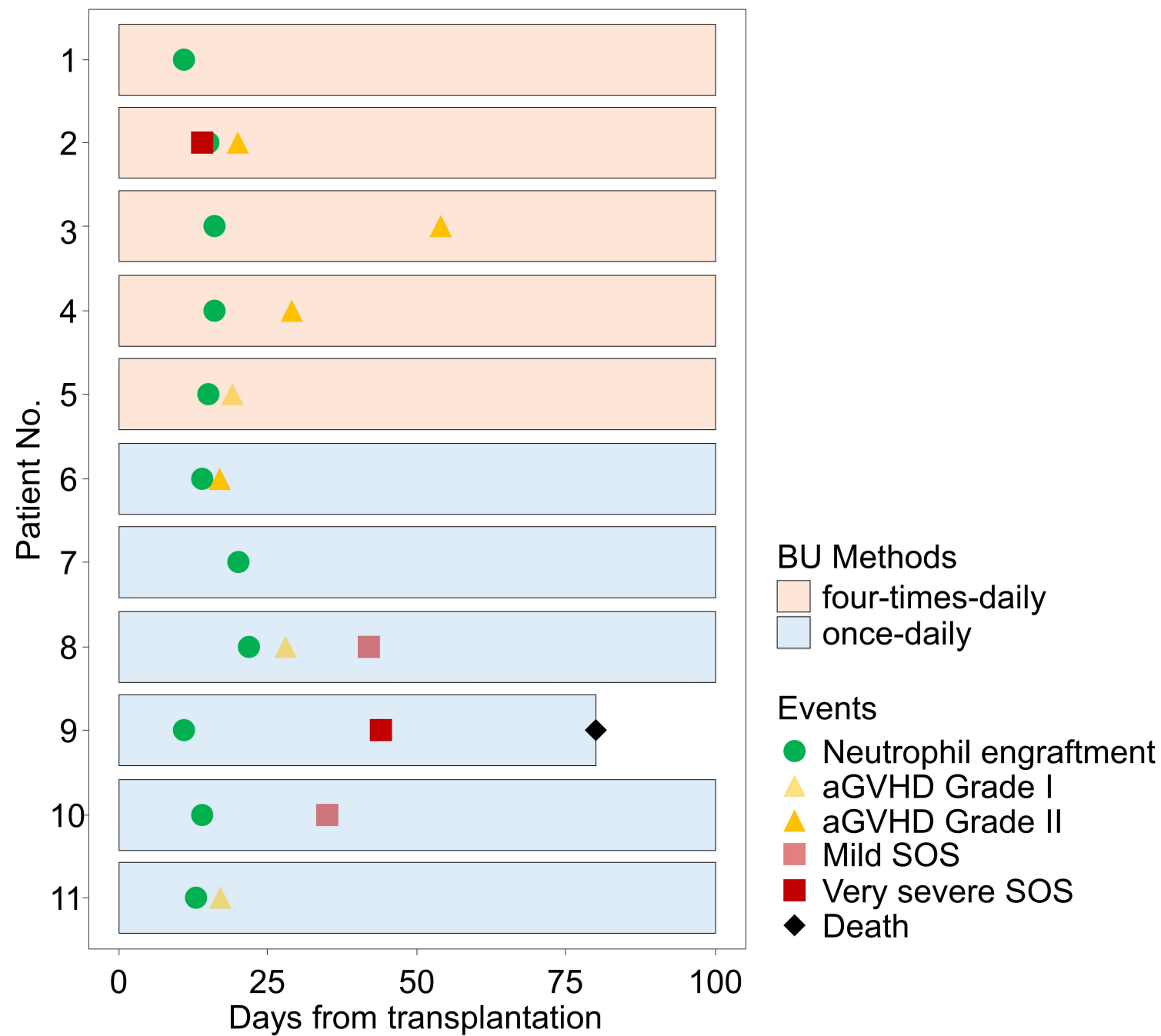
Figure 1 Progress in the acute phase after transplantation.

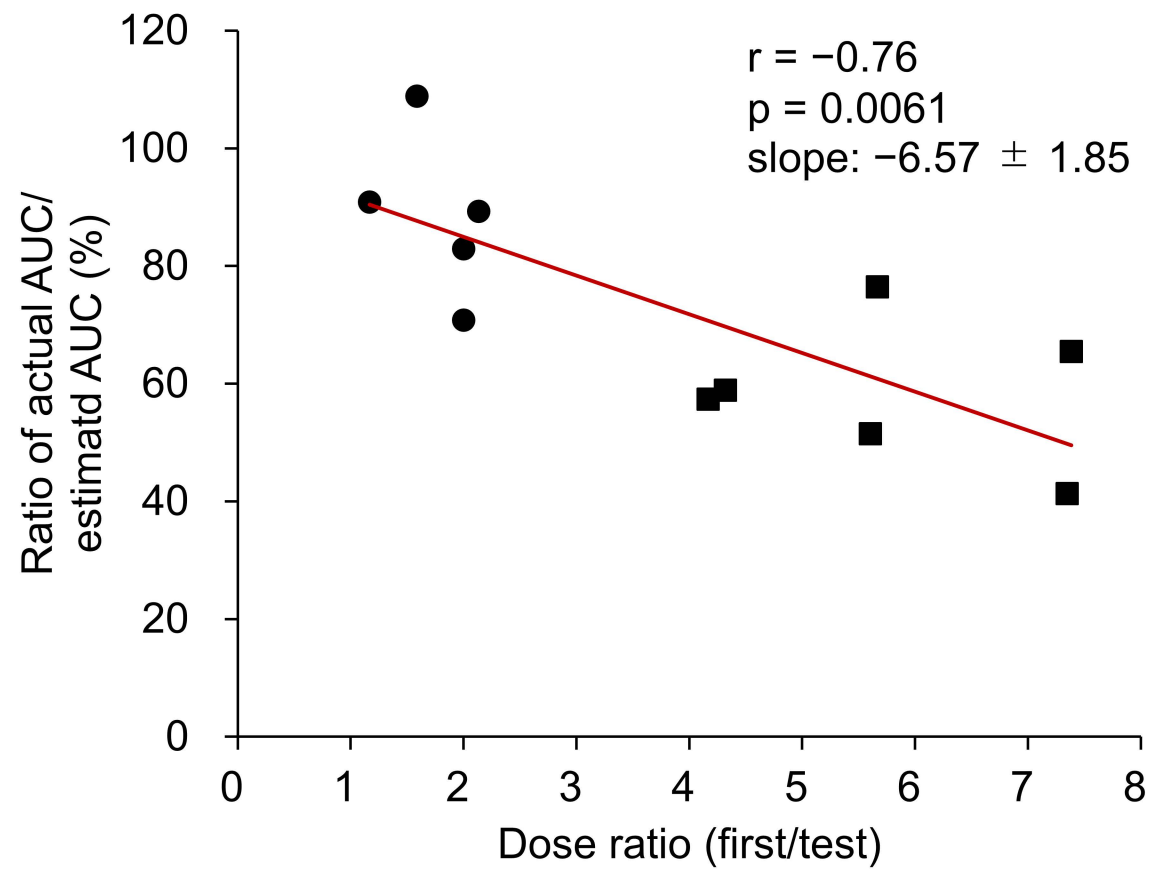
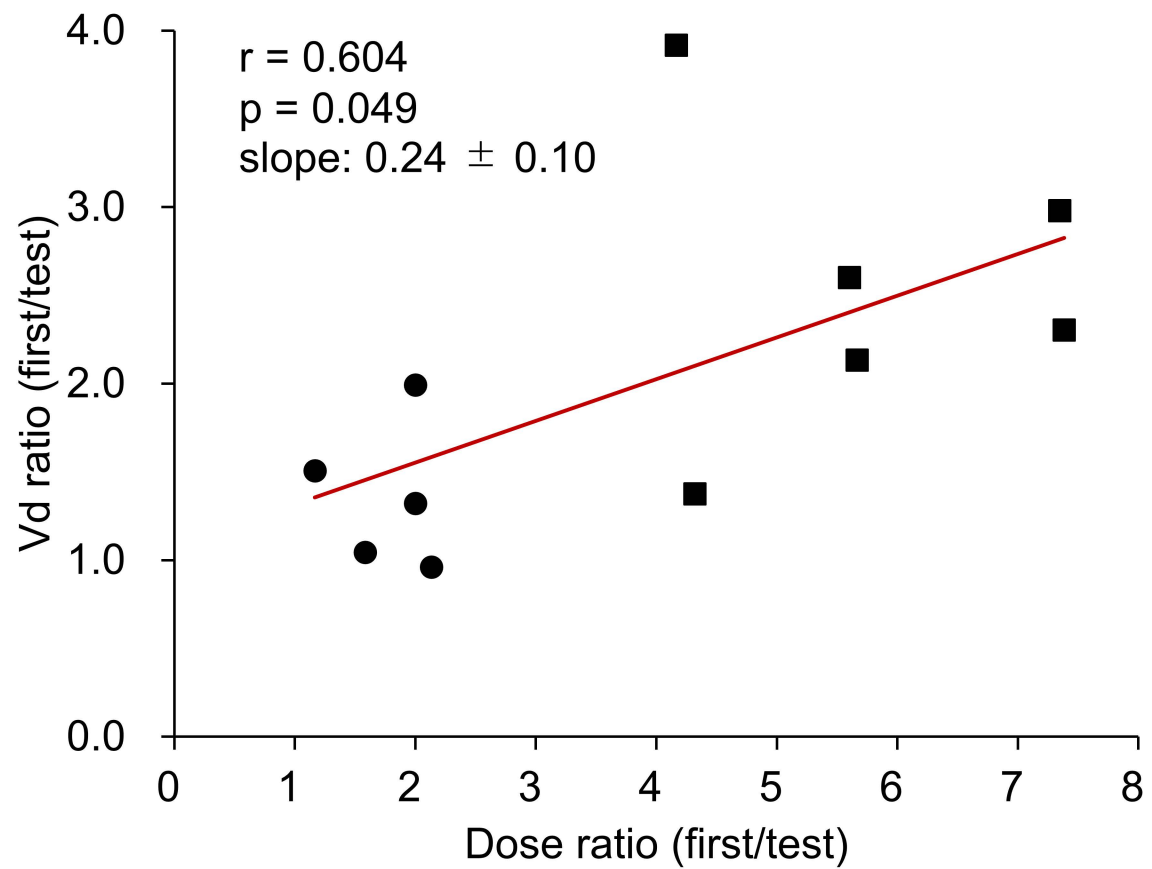
Figure 2 Correlation of the dose ratio for the test dose and first dose with Vd ratio (a) or coincident ratio of actual AUC for the first dose and estimated AUC for the test dose (b).

Circles represent four-times-daily method, and squares represent once-daily method.

AUC, area under the curve; Vd, distribution volume.

Figure 3 Trend in BU plasma concentration and urinary excretion for the first dose in once-daily method. BU, busulfan.





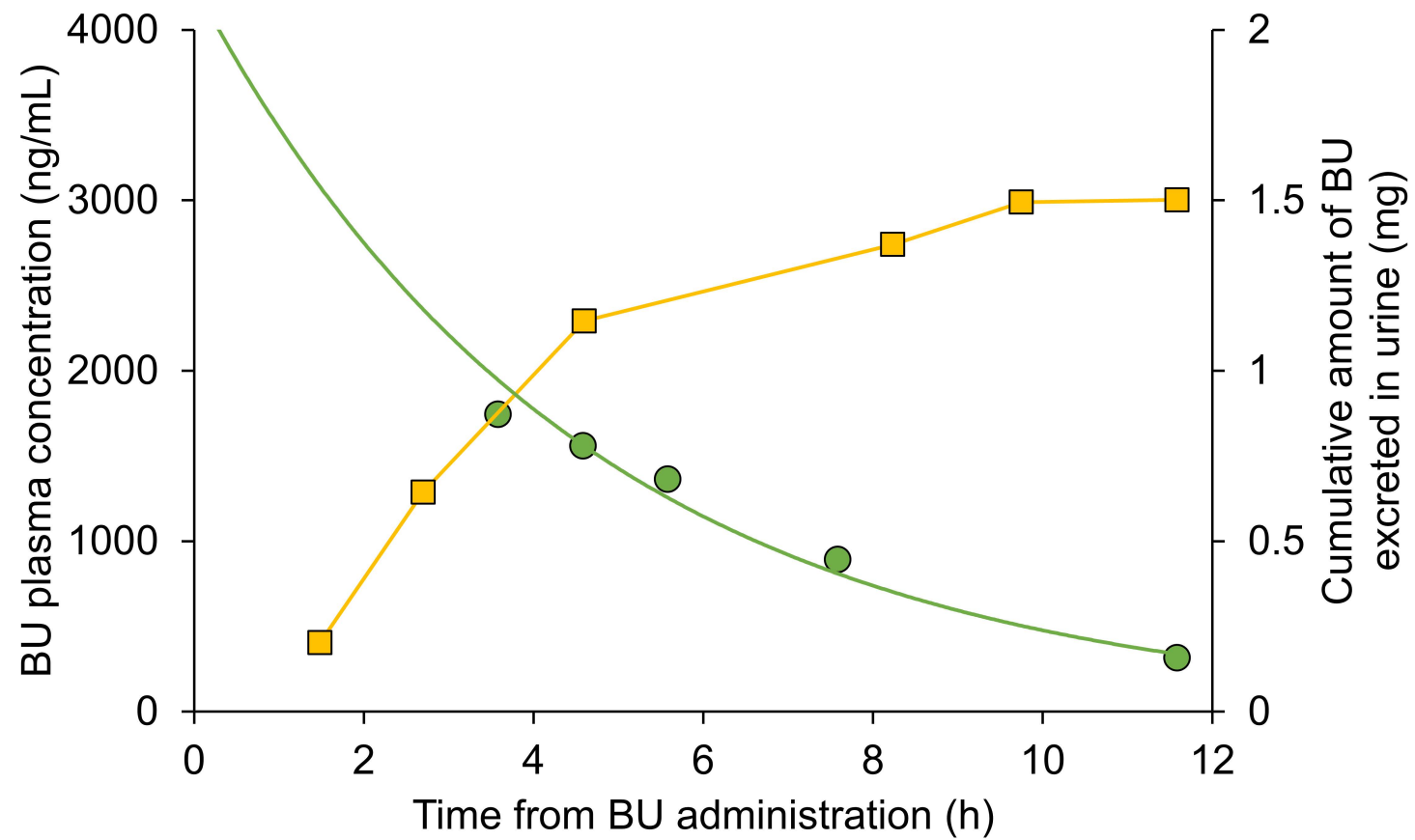


Table 1 Patients' characteristics and transplantation procedure

Total patients (n = 11)	
Sex (male/female)	9/2
Age at HSCT, year; median (range)	7.6 (0.4–11.8)
Weight, kg; median (range)	17.8 (6.5–46.3)
Malignant disease	
<i>Ewing sarcoma</i>	1
<i>Neuroblastoma</i>	1
Inborn errors of immunity	
<i>CGD</i>	2
<i>SAP deficiency</i>	2
<i>CD40 ligand deficiency</i>	1
<i>FHL</i>	1
<i>SCN</i>	1
Inborn errors of Metabolism	
<i>MPS</i>	2
Stem cell source	
<i>Autologous-peripheral blood</i>	2
<i>Unrelated allogeneic-cord blood</i>	3
<i>Unrelated allogeneic-bone marrow</i>	2
<i>Related-peripheral blood</i>	4
Conditioning regimen with BU (target AUC, mg*h/L)	
<i>Mel/BU (72)</i>	2
<i>CY/BU (75)</i>	2
<i>Flu/BU (65)</i>	1
<i>Flu/BU (65)/ATG</i>	3
<i>Flu/BU (65)/TBI 3 Gy/ATG</i>	1
<i>Flu/BU (65)/Alemtuzumab</i>	2
GVHD prophylaxis	
<i>sMTX+Tac</i>	5
<i>PTCY+Tac+MMF</i>	4

HSCT, hematopoietic stem cell transplantation; FHL, familial hemophagocytic

lymphohistiocytosis; CGD, chronic granulomatous disease; SCN, severe congenital

neutropenia; MPS, mucopolysaccharidosis; SAP, serum amyloid P component; BU, busulfan; Mel, melphalan; Flu, fludarabine; TBI, total body irradiation; CY, cyclophosphamide; GVHD, graft-versus-host disease; sMTX, short-term methotrexate; Tac, tacrolimus; PTCY, post-transplant cyclophosphamide; MMF, mycophenolate mofetil; ATG, anti-human thymocyte immunoglobulin.

Table 2 Engraftment data and toxicities

	Four-times-daily (<i>n</i> = 5)	Once-daily (<i>n</i> = 6)
Neutrophil engraftment days; median (range)	15 (11–18)	13.5 (11–22)
Graft failure	0	0
SOS	1	3
<i>Mild</i>	0	2
<i>Very Severe</i>	1	1
Seizures	0	1
aGVHD	4 (<i>n</i> = 4 for allogeneic)	3 (<i>n</i> = 5 for allogeneic)
<i>Grade I</i>	1	2
<i>Grade II</i>	3	1

SOS, sinusoidal obstruction syndrome; aGVHD, acute graft-versus-host disease.

Table 3 PK analysis

		Four-times-daily (<i>n</i> = 5)	Once-daily (<i>n</i> = 6)	p-value
Test dose	k_{el} (/h)	0.30 ± 0.06	0.31 ± 0.10	0.93
	CL (mL/min/kg)	3.62 ± 1.22	3.12 ± 1.11	0.54
	Vd (L/kg)	0.78 ± 0.15	0.73 ± 0.26	1.00
	AUC (mg*h/L/dose)	2.64 ± 0.25	3.01 ± 0.31	0.43
	Estimated cumulative AUC after dose adjustment by test (mg*h/L)	71.9 ± 8.34	65.6 ± 3.43	
First dose	Dose (mg/kg)	1.07 ± 0.24	3.45 ± 0.84	
	k_{el} (/h)	0.27 ± 0.08	0.22 ± 0.04	0.25
	CL (mL/min/kg)	4.23 ± 1.74	5.50 ± 2.22	0.33
	Vd (L/kg)	1.03 ± 0.21	1.71 ± 0.54	0.02
	AUC (mg*h/L/dose)	3.95 ± 0.54	9.69 ± 1.72	
	Actual cumulative AUC (mg*h/L)	63.20 ± 8.68	38.30 ± 7.70	
	Ratio of actAUC/estAUC (%)	88.60 ± 5.73	58.5 ± 5.24	<0.01

PK, pharmacokinetics; AUC, area under the curve; k_{el} , elimination rate constant; CL, clearance; Vd, distribution volume.