Two-point limited sampling strategy is necessary to estimate the area under the concentration-time curve for intravenous busulfan in infants and young children

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Abstract

Background: Therapeutic drug monitoring for busulfan is important to prevent adverse events and improve outcomes in stem cell transplantation. We investigated intravenous busulfan pharmacokinetics and evaluated the utility of limited sampling strategy (LSS) as a simple method to estimate the area under the concentration-time curve (AUC). Procedure: The study comprised 87 busulfan measurements in 54 children who received intravenous busulfan between August 2015 and May 2020. AUCs were calculated from 3–5 blood sampling points in each patient, and the correlation between AUC and plasma concentrations (ng/mL) at 1, 2, 3, 4, and 6 h after initiating busulfan infusion (C_1, C_2, C_3, C_4, and C_6, respectively). Results: By one-point sampling strategy, the most accurate predicted AUC was based on C_6 ($r^2 = 0.789$; precision, 11.0%) in all patients. The predicted AUC based on C_6 was highly precise ($r^2 = 0.937$; precision, 5.9%) in adolescent patients weighing > 23 kg, but the correlation was poor in infants and young children weighing [?]23 kg ($r^2 = 0.782$; precision, 11.4%). By two-point sampling strategy, the predicted AUC based on C_3 and C_6 showed the most favorable performance ($r^2 = 0.943$; precision, 6.4%), even in infants and young children, whereas the predicted AUC based on C_3 and C_6 was acceptable ($r^2 = 0.963$; precision, 5.7%). Conclusions: The AUC of busulfan can be predicted based on C_6 in adolescent patients. However, there was substantial inter-individual variation in busulfan pharmacokinetics in infants and young children, in whom two-point LSS was necessary for accurate AUC prediction.

Introduction

Therapeutic drug monitoring for busulfan is important for improving clinical outcomes in hematopoietic stem cell transplantation.[1] Busulfan exposure, expressed as the area under the concentration-time curve (AUC), is an important parameter for monitoring therapeutic efficacy and adverse events. A high busulfan AUC increases the risk of adverse events such as sinusoidal obstruction syndrome/hepatic veno-occlusive disease,[2] while a low busulfan AUC is associated with higher probability of graft failure or disease relapse.[1] Therefore, busulfan dosage is adjusted to a target AUC of 75–100 mg/L × h in myeloablative conditioning regimens.[3] Recent studies have demonstrated that reduced-intensity conditioning regimens with busulfan doses adjusted according to a target AUC of 45–65 mg/L × h are effective in chronic granulomatous disease and Wiskott-Aldrich syndrome.[4,5] Busulfan pharmacokinetics varies greatly among individuals,[6] and target AUC depends on the conditioning regimen and underlying disease; thus, it is clinically essential to calculate the busulfan AUC.

AUC is determined using the pharmacokinetic modeling software or the linear trapezoidal method; however, pharmacokinetic analyses require frequent blood sampling. Limited sampling strategies (LSSs) are used to estimate the AUC from a limited number of blood samples. Furthermore, LSSs permit the easy estimation

of AUC without using the pharmacokinetic modeling software, and the drug dosage can be immediately adjusted according to the blood concentrations, such as the trough value, which is of great importance in clinical practice. In the present study, we investigated the pharmacokinetics of intravenous busulfan and evaluated the utility of LSS as an accurate and simple method to estimate AUC in pediatric patients undergoing hematopoietic transplantation.

Methods

Patient selection

This retrospective study included 87 busulfan measurements in 54 consecutive children (age, 0.2–19.2 years) who received intravenous busulfan at the National Center for Child Health and Development from August 2015 to May 2020. The study was approved by the ethics committee of the National Center for Child Health and Development (approval number: 1964), and written informed consent was obtained from the guardian of each patient.

Busulfan dosing and pharmacokinetic analysis

Thirteen patients received body weight-based busulfan dosing (0.8–1.2 mg/kg)[7] without test dose. Forty-one patients received a test dose lower than the body weight-based busulfan dose, and the first dose was determined based on target AUC determined by the pharmacokinetics parameters obtained with the test dose. All patients were administered intravenous busulfan as a 2-h infusion every 6 h for four days, and pharmacokinetic analysis was performed based on the first dose. Blood samples at 3–5 time points per patient were collected at 1, 2, 3, 4, and 6 h from the start of busulfan infusion and processed using the dried blood spot method. Plasma busulfan concentrations were measured by liquid chromatography with tandem mass spectrometry.[8] Actual $AUC_{0-[?]}$ values were calculated from the data on plasma concentrations at all time points by applying the one-compartment model using the Phoenix(r) WinNonlin 7.0 pharmacokinetic analysis software (Certara LP, Princeton, NJ, USA).

Evaluation of LSSs

First, the regression equation was calculated from individual busulfan concentrations at each sampling point and the actual $AUC_{0-[?]}$ obtained from the pharmacokinetic modeling software. Second, the predicted $AUC_{0-[?]}$ was calculated from the regression equation and individual busulfan concentrations. Finally, the relationship between the predicted $AUC_{0-[?]}$and actual $AUC_{0-[?]}$ was evaluated using Pearson's correlation coefficient (r^2). LSSs using two or three busulfan sampling points were developed by multiple regression analysis. The precision of the predictive performance of busulfan AUCs was assessed by calculating the mean absolute percentage error (MAPE). Additionally, subgroup analysis was conducted based on body weight (<9, 9–16, 16–23, 23–34, and >34 kg). To verify the utility of LSSs in the present study, the predicted $AUC_{0-[?]}$ was calculated using the developed LSS and its predictive performance was evaluated in seven patients in a cohort separate from the first cohort that was used to develop the LSS. All statistical analyses were performed using SPSS Statistics ver.23 (SPSS IBM Japan, Tokyo, Japan).

Results

Patient characteristics

A total of 87 busulfan measurements in 54 patients were included. The patient characteristics are shown in Table 1. There were 45, 87, 75, 84, and 82 blood samples at 1, 2, 3, 4, and 6 h from the start of busulfan infusion, respectively. The median actual $AUC_{0-[?]}$ was 773 (range, 331–1631) μmol*min/L.

Development of the LSS

The relationship between the predicted and actual $AUC_{0-[?]}$ values using the one-point sampling strategy with plasma busulfan concentrations (ng/mL) measured at 1, 2, 3, 4, and 6 h after the initiation of busulfan infusion (C1, C2, C3, C4, and C6, respectively) is shown (Figure 1). The most accurate predicted $AUC_{0-[?]}$ in all patients using the one-point sampling strategy was based on C_6 (r^2 = 0.789; MAPE, 11.0%). The

multiple regression analysis of the predicted $AUC_{0–[?]}$ values based on C_3, C_4, and C_6 using the two-point and three-point sampling strategies revealed that the one-point sampling strategy was highly precise. By the two-point sampling strategy, the $AUC_{0–[?]}$ predicted based on C_3 and C_6 exhibited the most favorable performance ($r^2 = 0.943$; MAPE, 6.4%). The $AUC_{0–[?]}$ predicted based on the three-point sampling strategy using C_3, C_4, and C_6 showed excellent predictive performance when compared with the actual $AUC_{0–[?]}$ ($r^2 = 0.955$; MAPE, 5.9%) (Table 2).

The predictive performance of busulfan AUC based on body weight are shown in Table 3. In adolescent patients (body weight > 23 kg), the $AUC_{0–[?]}$ predicted based on $C_6$was highly precise ($r^2 = 0.937$; MAPE, 5.9%); however, this correlation was poor in infants and young children weighing [?]23 kg ($r^2 = 0.782$; MAPE, 11.4%). By the two-point sampling strategy using C_3 and C_6, the predicted $AUC_{0–[?]}$ was acceptable even for infants and young children ($r^2 = 0.963$; MAPE, 5.7%) and was as accurate as the $AUC_{0–[?]}$based on C_6 in adolescent patients ($r^2 = 0.937$; MAPE, 5.9%).

Validation of the LSS

The accuracy of the predicted $AUC_{0–[?]}$ by one-point and two-point sampling strategies was determined in a cohort of seven patients who were not included in the development of the LSS (Table 4). All seven patients weighed [?]23 kg. By the one-point sampling strategy, the predicted $AUC_{0–[?]}$ had more than 15% errors compared to the actual $AUC_{0–[?]}$ in four patients. In contrast, none of the patients had more than 15% error by the two-point sampling strategy.

Discussion

In the present study, AUC prediction based on C_6 was the most accurate one-point blood sampling strategy. Watanabe *et al* .[9] reported that the AUC of busulfan was best predicted by C_6 in pediatric hematopoietic stem cell transplant recipients, supporting the results of the present study. The reason for the poor predictive performance of AUC based on C_1 could be because the individual differences in the absorption phase were smaller than in the elimination phase due to the intravenous administration of busulfan. C_2 represents the blood sampling immediately after the end of busulfan infusion and may reflect the sampling of the distribution phase. Therefore, the blood concentrations may significantly change within a few minutes, which might explain the weaker relationship between the predicted AUC based on C_2 and the actual AUC.

In the present study, the predictive performance of AUC based on C_6 was lower ($r^2 = 0.789$) than that reported by Watanabe *et al.* [9]($r^2 = 0.929$). More than 60% of all patients weighed [?]23 kg in that study, whereas in our study, 24% of the patients weighed [?]23 kg, which might have reduced the accuracy of predicted AUC based on C_6. In fact, from the predictive performance of AUC performance by body weight, the predicted AUC based on C_6 was highly precise in adolescents.

In infants and young children, the accuracy of AUC predicted by one-point sampling with C_6 was poor. Kishimoto *et al* .[10] reported that errors in AUC prediction based on C_6 were higher in young children and infants using the Monte Carlo simulation in the population pharmacokinetic model. Furthermore, McCune *et al* .[6] reported that the accuracy of prediction for busulfan to reach the therapeutic range was lower in patients younger than five years than in adolescent patients using the population pharmacokinetic model. Therefore, in early childhood, the busulfan pharmacokinetics significantly varies among individuals, and predicting AUC by the one-point sampling strategy may not be sufficient. In the present study, the AUC prediction based on C_3 and C_6 using the two-point sampling strategy was acceptable even for infants and young children. In the validation cohort, more than half of the patients had more than 15% errors with the one-point sampling strategy compared to the actual AUC. Errors >15% are a deviation from the guidelines on bioanalytical method validation in pharmaceutical development,[11] which states that the accuracy and precision should be within ±15% deviation of the theoretical concentration. Thus, in infants and young children, the AUC should be predicted using the two-point sampling strategy.

In the one-point sampling strategy, the LSS equation for AUC was 2.866 C_6 + 108.9 in adolescent patients. Consequently, the target C_6 was 360–500 ng/mL for myeloablative conditioning (target AUC, 75–100 mg/L

× h) and 200–300 ng/mL for reduced-intensity conditioning (target AUC, 45–65 mg/L × h). In contrast, in the two-point sampling strategy, the target C_3 depended on C_6 and target AUC. It should be noted that the accuracy of the predicted AUC by LSS will decrease if the blood sampling time is significantly different from the correct sampling time. Thus, it is important to pay close attention to blood sampling times.

Concomitant medications that may affect the busulfan pharmacokinetics should be considered in the interpretation of our findings. Kangarloo*et al* .[12] reported that phenytoin, an anticonvulsant agent, increased the busulfan clearance. In the present study, levetiracetam or clonazepam were used as anticonvulsants, which do not affect the busulfan pharmacokinetics.[13,14]Therefore, the anticonvulsant agent did not have a significant effect on the busulfan pharmacokinetics in the present study.

The present study also has certain limitations. It remains unclear why busulfan pharmacokinetics varies significantly in infants and young children. Polymorphisms in glutathione S -transferase (GST)[15] affect busulfan pharmacokinetics. Additionally, age-dependent variations in intrinsic busulfan clearance were reported to be associated with higher GST activity[16]; thus, differences in the GST expression might explain individual differences in busulfan pharmacokinetics. Further investigation is warranted to establish the parameters for personalized administration of busulfan.

In conclusion, the AUC of busulfan could be predicted based on C_6 in adolescent patients. However, for infants and young children, there was substantial inter-individual variation in busulfan pharmacokinetics and the two-point sampling strategy was necessary for accurate AUC prediction.

Conflicts of interest

The authors have no conflicts of interest.

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Legends

Figure 1. Predictive performance of busulfan AUC using the one-point sampling strategy. Relationship between actual $AUC_{0-[?]}$ calculated by the pharmacokinetic modeling software and predicted $AUC_{0-[?]}$ calculated by the limited sampling strategy based on plasma busulfan concentrations at 1 (C_1), 2 (C_2), 3 (C_3), 4 (C_4), and 6 (C_6) h after initiating busulfan infusion is shown.

AUC, area under the concentration-time curve

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