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SHORT COMMUNICATION

Population pharmacokinetic study of a test dose oral busulfan in Japanese adult patients undergoing hematopoietic stem cell transplantation

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Abstract
Purpose The aim of this study is to determine the population pharmacokinetics of oral busulfan in Japanese adults.
Methods We previously underwent a clinical trial involving the dose adjustment of oral busulfan depending on the individual pharmacokinetics using a test dose in hematopoietic stem cell transplantation recipients. Seventy-one Japanese patients aged from 16 to 67 years were enrolled. After taking oral busulfan 0.5 mg/kg as a test dose, blood samples were collected at five time points from each patient. Busulfan concentrations were measured by high-performance liquid chromatography, and the individual parameters were estimated by using the nonlinear mixed effects model computer program. A one-compartment model with first-order absorption was sufficient to describe the concentration–time profile.
Results The final pharmacokinetic parameter were the clearance (CL/F) = 0.153 L/h/kg, distribution volume (Vd/F) = 0.695 L/kg, and absorption rate constant (ka) = 2.39 h^{-1}. The inter-individual variabilities in CL/F, Vd/F and ka were 25.9, 26.2, and 111.8%, respectively, and the residual variability was 12.1% as the coefficient of variation.
Conclusion We developed a population pharmacokinetic model of oral busulfan in Japanese adults. The final population model was implemented into the program excel, leading to an easy and proper therapeutic monitoring of oral BU by using small number of samples.

Keywords Busulfan · Hematopoietic stem cell transplantation · Japanese adults · Population pharmacokinetics · Therapeutic drug monitoring

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Introduction

Busulfan (BU) is an alkylating agent generally used for a conditioning regimen of hematopoietic stem cell transplantation (HSCT). The standard dose of BU is 1 mg/kg body weight administered orally every 6 h. Therapeutic effects of BU are related to the area under the plasma concentration–time curve (AUC) or the average plasma concentrations at a steady state (Css) [1]. Excessively high BU AUC is associated with an increase in treatment-related toxicities such as hepatic veno-occlusive disease (VOD) [2], while low BU Css is related to a high relapse rate in patients with chronic myeloid leukemia [3] and a graft rejection in children [4, 5]. We previously investigated the

pharmacokinetics (PK) of BU in patients who received 1 mg/kg of oral BU for allogeneic HSCT and found that BU Css varied widely from 745 to 2,422 ng/mL and that excessively high BU Css level was associated with the development of hepatic VOD, a life-threatening complication associated with HSCT [6].

To minimize toxicities and improve clinical outcomes, BU dose adjustment according to the individual BU PK has been extensively investigated in Caucasians [7, 8]. We then underwent a prospective clinical study involving adjusting the BU dose in Japanese adult patients [9]. After taking a 0.5 mg/kg test dose of oral BU, individual BU PK parameters were analyzed, and the adjusted BU dose was calculated to achieve a target BU Css of 850 ng/mL. Actual BU concentrations were significantly correlated with expected BU concentrations, and the predictability of BU Css was 103 ± 19%. The incidence of toxicity excluding oral mucositis was low, and there was no regimen-related toxicity-associated mortality. It showed that our method to calculate individual BU doses using a test dose allowed reliable prediction of the actual BU Css and successful clinical outcomes by reducing early adverse events in HSCT recipients treated with oral BU-containing conditioning regimens. However, there were some patients unable to achieve the target range of Css. There is wide inter-individual variability and inter-dose variability in oral BU PK, and population PK is helpful to identify factors affecting the variability of PK. In the previous study, we used the population parameter examined in Caucasians [10], because there was no study for Japanese.

BU is metabolized mainly in the liver through conjugation with glutathione by glutathione S-transferase (GST) [11]. In patients with β-thalassemia major treated with BU plus cyclophosphamide for the conditioning of HSCT, the incidence of hepatic VOD was significantly higher in patients with the GST M1-null genotype in comparison with the GST M1-positive genotype [12]. The GST M1-null genotype is observed in 55% of Caucasians, 33% of Black subjects, and 20% of Amazonian Indians, indicating the ethnic variation in the prevalence of GST M1 genotype [13]. The influence of the polymorphism of GST A1, the most active form of GST, was investigated in 12 patients treated with high-dose oral BU. When compared with 9 patients who have wild-type GSTA1*A/*A, three patients having heterozygous variants GSTA1*A/*B have significantly lower elimination constant [14]. The ethnic differences were also reported in the prevalence of GST A1 genotype [15]. It suggests that the BU metabolism influenced by the polymorphism of GST genotypes has a genetic basis. We therefore estimated a population BU PK in Japanese adults by using the nonlinear mixed effects model (NONMEM) computer program.

Materials and methods

Patients

This study was conducted as an accompaniment of the prospective clinical trial investigating an individual dose adjustment of oral busulfan using a test dose [9]. All consecutive patients undergoing allogeneic HSCT with BU-based conditioning regimens at nine institutes in Japan were enrolled in this study. A total of 71 Japanese patients aged from 16 to 67 (median 44) years, 46 men and 25 women, were entered in this study. Indications for HSCT were 35 of acute myeloid leukemia, 13 of myelodysplastic syndrome, 4 of each acute lymphocytic leukemia and malignant lymphoma, 3 of each chronic myeloid leukemia and adult T cell leukemia, 2 of each prolymphocytic leukemia, multiple myeloma, and myelofibrosis, and 1 of each Ewing sarcoma, histiocytic sarcoma, and malignant thymoma. This study was approved by the institutional review board.

Blood sampling and measurement of plasma BU concentrations

After achieving an informed and written consent, a test dose of BU 0.5 mg/kg of ideal body weight was administered orally about a week prior to the HSCT preparative regimen. None of the patients were treated with any concomitant drugs such as anticonvulsants and antimicrobials that might have influenced the BU PK. Blood samples were collected for PK analysis in heparinized tubes at 30, 60, 120, 240, and 360 min after the oral BU administration. After centrifuged at 3,000g for 10 min, plasma samples were separated and stored at −20°C until analysis. Plasma concentrations of BU were measured by a high-performance liquid chromatography (HPLC, Shimazu, Japan) as previously described [16].

Population pharmacokinetic analysis

AUC was computed with the trapezoidal method and Css was calculated from AUC as follows: Css (ng/mL) = AUC (ng h/mL)/dosing interval (h).

The data were analyzed by using NONMEM program (version V, level 1.1) on a Hewlett Packard computer (HP Apollo 9000 model 712/60; Hewlett Packard, Palo Alto, CA, USA). A one-compartment model with a first-order absorption (PREDPP program, subroutine ADVAN2 and TRANS2), not post hoc, was used to depict the plasma concentration–time course of BU. The oral clearance (CL/F), distribution volume (Vd/F), and absorption rate constant (ka) were also determined.

The inter-patient variability in the three fundamental PK parameters (CL/F, Vd/F, and ka) was modeled with proportional error according to the following equation:

$$P_j = P'_j(1 + \eta_j) \quad (1)$$

where P'_j represents the mean population parameters (CL/F, Vd/F, and ka), P_j represents the individual parameters for patients *j,* and η_j is an independently distributed random variable with mean zero and variance ω^2.

The residual variability was also modeled with proportional error according to the following equation:

$$C_{ij} = C'_{ij}(1 + \varepsilon_{ij}) \quad (2)$$

where C_{ij} is the measured plasma concentration collected at time i from patient j; C'_{ij} is the corresponding predicted plasma concentration. ε_{ij} is the residual variability term, representing independent identically distributed statistical error with mean zero and variance σ^2_E for plasma concentrations.

A preliminary analysis was conducted by using NONMEM to estimate the parameters of the basic model (i.e. no covariates). The objective function values (OFV) were analyzed before and after adopting one covariate to the model, and the changes in OFV were compared. The difference in the OFV values was approximately distributed when the chi-square test was applied, and the degree of freedom was assumed to be equal to the difference in the number of parameters between the two models. The difference in OFV of at least 6.63, corresponding to $P < 0.01$, was considered statistically significant.

Results

A total of 346 plasma samples were collected. Plasma concentration–time profiles of BU after a test dose are shown in Fig. 1a. A preliminary analysis was conducted by permitting NONMEM to estimate the parameters of the basic model (i.e. no covariates). Each candidate covariate, such as total body weight, body surface area, age and gender, was added to the basic model, and the change in the OFV was analyzed. Both body weight and body surface area were significant covariates affecting clearance; however, the influence of body weight was greater than that of body surface area (the difference in OFV was 70.77 and 64.05, respectively). Gender and age did not significantly improve the estimate of clearance.

The final pharmacokinetic parameters were CL/F = 0.153 L/h/kg, Vd/F = 0.695 L/kg, ka = 2.39 h^{-1}. The minimum value of the objective function in the NONMEM run was −1,274.634, and the objective function difference from the value of the basic model was 70.77. The estimated population PK parameters of BU are shown in Table 1.

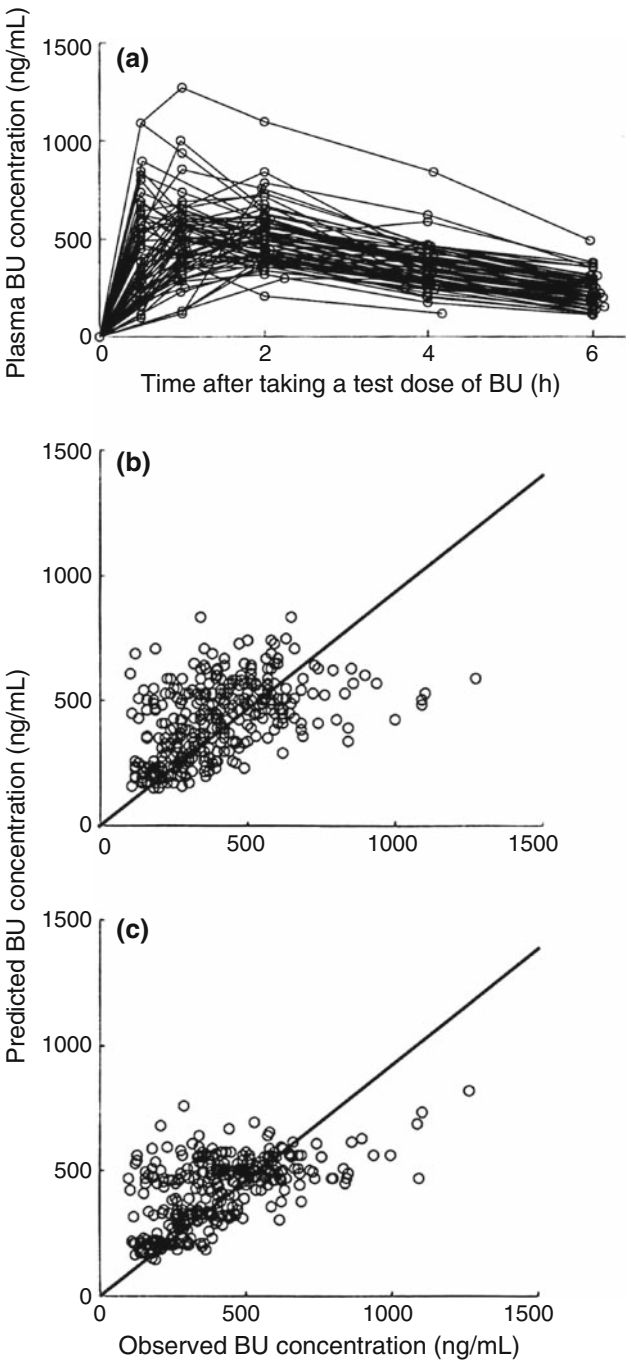

Fig. 1 Plasma concentration–time profiles of oral busulfan following a single administration of 0.5 mg/kg as a test dose in 71 patients (**a**). Plots of the observed BU concentration versus BU concentration predicted by the basic model (**b**) and the final model (**c**)

The inter-individual variabilities in CL/F, Vd/F, and ka were 25.9, 26.2, and 111.8%, respectively, and the residual variability was 12.1% as the coefficient of variation.

Plots of the observed BU concentrations versus the concentrations predicted by the basic and final models are shown in Fig. 1b, c, respectively. The predictive

Table 1 Final estimates of population pharmacokinetic parameters

	Estimated values	95% confidence interval
θ_1	0.153	0.142–0.164
θ_2	0.695	0.641–0.749
θ_3	2.39	2.05–2.73
ω_{cL} (%)	25.9	20.4–30.5
ω_{vd} (%)	26.2	20.2–31.2
ω_{ka} (%)	111.8	71.6–140.9
σ (%)	12.1	10.5–13.5

θ_1 CL (L/kg/h), θ_2 Vd (L/kg), θ_3 ka (h^{-1})

ω inter-individual variability

σ residual variability

performance of the basic and the final parameters were evaluated by the mean prediction error (ME) and the mean absolute prediction error (MAE) [17]. The ME in the basic and final models were 23.9 ± 177.0 and 14.8 ± 157.8 ng/mL, respectively. The MAE in the basic and final models were 131.3 ± 120.7 and 114.6 ± 109.3 ng/mL, respectively. The performance (bias and precision) of the final population parameters was equivalent to that of the basic population parameters.

Discussion

We carried out the BU population analysis in Japanese adult patients aged from 16 to 67 years, and found that the PK parameters as follows; CL/F = 0.153 L/h/kg, Vd/F = 0.695 L/kg, and ka = 2.39 h^{-1}. Sandstrom et al. reported that the CL/F, Vd/F, and ka were 0.149 L/h/kg, 0.64 L/kg and 1.68 h^{-1}, respectively [10]. It indicated that CL/F and Vd/F of Japanese were not different from those of Caucasians, while ka was about 1.5-fold larger than that of Caucasians, and the inter-individual variability of ka was 111.8%. It is shown that the CL/F is correlated with age in a pediatric population, but ka is not associated with age [18]. The type of drug formulation generally influences ka. However, the formulation of oral BU is similar when given as a conditioning of HSCT. The factor responsible for the difference in ka between Japanese and Caucasian is unclear.

We next investigated the effect of covariates on BU PK parameters. It shows that the total body weight is a determinant of CL/F and Vd/F. Recently, Nakamura et al. [19] reported that population pharmacokinetics in young Japanese children aged from 2 months to 11 years. They showed that oral BU clearance was correlated with age as well as serum aspartate transaminase (AST) and type of the underlying disease. CL/F is low at early infancy, then increase to a maximum at approximately 2 years of age, and thereafter decreases. We found that CL/F was affected by neither age nor AST in adult patients aged from 16 to 67 years, although the influence of age in pediatrics was undetermined in the present study. They also demonstrated that malignant disease was a negative influencing factor for CL/F when compared with an inherited disorder. Since all the patients entered to our study were having malignancies, we were unable to evaluate the influence of disease types on BU clearance.

It is known that BU PK is influenced by other drugs using a common metabolic pathway such as phenytoin, itraconazole, and metronidazole. The relationship between the BU Css and toxicity are expected to be altered when BU is combined with other agents such as cyclophosphamide, thiothepa, or melphalan as a conditioning regimen for HSCT [18]. Since none of these drugs that might have influenced the BU PK was given when a test dose of BU was administered, it is conceivable that the interaction of BU with other agents is not associated with the variability of BU PK observed in this study.

Intravenous BU (IV BU) has recently been approved as a conditioning agent for allogeneic HSCT and autologous HSCT in Ewing sarcoma and neuroblastoma by the Ministry of Health, Labor and Welfare in Japan. The inter-individual variability of ka can be resolved by using IV BU instead of oral BU. However, variability of CL/F is unsolvable, even if BU is administered intravenously [20]. We and others previously showed that individual dose adjustment of oral BU allowed reliable prediction of the actual BU Css and successful clinical outcomes in HSCT recipients [7–9]. It seems that oral BU is a treatment of choice in patients who receive high-dose BU for a conditioning regimen of HSCT.

In conclusion, we developed a population PK model of oral BU in Japanese adults. The final population model was implemented into the program Excel. Clinical application of this parameter may allow patients to achieve an easy and a proper therapeutic drug monitoring by using small number of samples when high-dose BU was given as a conditioning regimen for HSCT.

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