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Mini Test Dose of Intravenous Busulfan (Busulfex®) in Allogeneic Non-Myeloablative Stem Cell Transplantation, Followed by Liquid Chromatography Tandem-Mass Spectrometry

Ivan Spasojevic, PhD^{1,2,*}, Ligia R. S. da Costa, MD³, Mitchell E. Horwitz, MD³, Gwynn D. Long, MD³, Keith M. Sullivan, MD³, John P. Chute, MD³, Cristina Gasparetto, MD³, Ashley Morris, PharmD³, Nelson J. Chao, MD³, and David A. Rizzieri, MD^{3,*}

¹Department of Medicine, Division of Oncology, Duke University Medical Center, Durham, NC

²PK/PD Bioanalytical Core Resource of Duke Cancer Institute, Duke University Medical Center, Durham, NC

³Department of Medicine, Division of Cellular Therapy, Duke University Medical Center, Durham, NC

INTRODUCTION

Busulfan (Bu) has been used for almost 3 decades in the conditioning of hematopoietic stem cell transplant. Bu has a very diverse gastrointestinal absorption which in association with its emetic effect in high doses makes inter- and intra-individual oral bioavailability and thus systemic exposure very variable and difficult to predict (1, 2, 3). If the systemic exposure is below the therapeutic window there is an increased risk of lack of engraftment (4) and, in chronic myelogenous leukemia (CML) patients, disease relapse (5), while overexposure results in an increased incidence of high-dose related toxicities like hepatic veno-occlusive disease (6, 7). It is important to note that “busulfan concentration-response relationships are regimen-, age- and disease-dependent”, as emphasized by McCune *et al* (8). Therapeutic dose monitoring (TDM) gained an important role in the setting of oral Bu dose as it became clear that the level of systemic exposure influenced the toxicity, engraftment and relapse rates. To give an idea of the importance of TDM, a test dose prior to starting the conditioning regimen with home-administered oral Bu in 153 patients evidenced that as many as 68% of them would have drug exposures out of the therapeutic window (900–1,500 µM·min) with the standard dose of 1mg/kg of ideal body weight every 6 hours (9).

An intravenous (i.v.) formulation was developed to overcome these issues and avoid the utilization of TDM. However, in a series of 6 studies using intravenous Bu in adults, 72 to 91% of the patients achieved the target AUC without dose changes, while 10 to 82% of children achieved it in a series of 5 studies with the intravenous drug (10). Clearly, a need for TDM even when i.v. is used still persists. Many different strategies using single or repeated sampling in different points of the therapy were proposed to improve TDM results (10). However, since TDM can only be useful if Bu determinations are timely delivered and many transplant centers do not have a facility to perform Bu determination, new approaches using pharmacokinetic analysis of a *sub-therapeutic test dose administered a few days earlier* to predict the ideal dose were proposed. In one such study, 7 patients received a test

*Corresponding authors: Ivan Spasojevic, Duke University Medical Center, Durham, NC, ivan.spasojevic@dm.duke.edu. David Rizzieri, Duke University Medical Center, Durham, NC, david.rizzieri@dm.duke.edu.

dose of 0.8 mg/kg, followed by 0.8 mg/kg/dose every 6 hours for 15 doses (11). Bu concentrations were obtained and mean drug clearance was determined after the test, first and 13th doses. The utilization of a test dose demonstrated bioequivalence (<10% variability) with the first high-dose Bu dose, but not with the 13th dose (>20% variability). However, when the same 0.8 mg/kg test dose assay was compared to the intravenous Bu in continuous infusion for 90 hours in other 7 patients, they showed similar clearances (12). The next step was to examine the ability of the test dose clearance to base the calculation of the 90 hour continuous infusion dose (13). All 4 patients tested had AUCs within the desired range and no adjustments were required after 18 hours. More recently, Beri *et al.* (14) used a 0.8 mg/kg i.v. test dose (1/4 of the standard daily dose). Without the test dose, 23% of the 17 patients would have fallen below and 12% above the therapeutic range. With the test dose, no patients had a sub-therapeutic AUC and only 12% had an AUC above the therapeutic window.

In this work, in order to further reduce the unnecessary exposure and adverse effects of the test dose process, we investigated bioequivalence of a *very low* sub-therapeutic test dose (1/10 of the standard daily dose) of i.v. Bu (Busulfex®). For this purpose we developed a sensitive and fast liquid chromatography tandem-mass spectrometry (LC-MS/MS) method which allowed us to measure Bu in a wide concentration range required in the study.

EXPERIMENTAL

Utility of the LC-MS/MS assay and bioequivalence of the test vs therapeutic regimens were investigated in the “Myeloid Cohort” (patients with a high chance of progressive myeloid diseases, marrow failure syndromes or myeloproliferative disorders) of the ongoing “Efficacy of T-Cell Depleted Allogeneic Non-Myeloablative Stem Cell Transplantation” study. The treatment flow chart is presented in Table 1. During both test and therapeutic Bu regimens, plasma was collected at pre-dose, 15 min, 1, 2, 4, and 24 hrs. To 100 µL of plasma sample, 100 ng of busulfan-d8 (internal standard) was added. After extraction of analytes by 1 mL of ethylacetate, evaporation of the solvent and reconstitution in LC mobile phase, 50 µL of the sample was injected into the LC-MS/MS system. Solid-core Kinetex C18 50mm × 4.6 mm, 2.6 µm analytical column and 4mm × 2 mm Kinetex C18 guard column were used for chromatographic separation. Mobile phase A: 10 mM ammonium acetate, pH 4.5 by acetic acid, 5% methanol. Mobile phase B: methanol (LCMS grade). Elution gradient: 0 min, 0% B; 0–1.5 min, 0–80% B; 1.5–2 min, 80% B, 2–2.2 min, 80–0 % B. Applied Biosystems/SCIEX API 4000 tandem-mass (MS/MS) instrument in positive electrospray mode was used for detection. Parameters: curtain gas = 30, gas1 = 40, gas2 = 30, ion spray potential = 4500 V, ionization chamber temp. = 500 °C; declustering potential = 36 V, entrance potential = 10 V, collision energy = 17, collision cell exit potential = 14 V. Calibration samples in 1.6 ng/mL – 5 µg/mL range of pure Bu in pooled healthy volunteer plasma were run along the study samples. LLOQ of the method (80% accuracy criterion) was 1.6 ng/mL. Pharmacokinetic (PK) parameters were calculated by non-compartmental approach using WinNonlin software.

RESULTS and DISCUSSION

Presented here are results from 3 patients infused on consecutive days with 18 min long test dose and 180 min long therapeutic dose. Figure 1A shows a typical plasma Bu conc./time profile obtained. Figure 1B is a semi-log (log conc. vs time) plot illustrating equal terminal slopes (elimination rate, clearance) for both test and therapeutic doses. This “day-before, 24-hr PK test” approach enabled for accurate assessment of the full PK profile and exposure (AUC). Obtained AUC_{TEST}/AUC_{THERAP} ratio for patients 1, 2, and 3 was 7.5, 8.5, and 7.7,

respectively. Table 2 lists $AUC_{\text{therap}}(\text{PREDICTED})$ values calculated by least-square regression analysis of $AUC_{\text{therap}}(\text{MEASURED})$ vs $AUC_{\text{test}}(\text{MEASURED})$ data (**Plot 1**).

LC-MS/MS method has been published (24) but for our needs (measurement after both therapeutic and 1/10 dosing regimens) it was necessary to expand the concentration range and to utilize a stable isotope as internal standard (Bu-d8) to provide for method accuracy and robustness needed for TDM. The assay proved to be optimal in terms of concentration range (1.6 ng/mL – 5 µg/mL), throughput (4 min per analytical run), and small sample size (100 µL plasma). Stable-isotope internal standard (Bu-d8) co-elutes with the measured analyte (Bu) and compensates for variable matrix effects of human plasma (genetic variability, diet, disease stage, organ function and other drug treatments) and can be used in an ambulatory setting. This is key as the test dosing is to be performed in varied conditions and populations in an outpatient setting where drug measurement reliability is critical.

Non-compartmental PK analysis (AUC) suggests: (a) low inter-patient variability in both test and therapeutic doses (Table 2) and (b) promising merit of the 1/10 test dose in predicting exposure by the first therapeutic dose (Table 2). This very low test dose is attractive for minimizing toxicity and allowing TDM therapy for this agent. The results from the limited number of samples are very convincing and encouraging but a study with a larger number of subjects is needed for verification of the safe first-dose prediction.

References

1. Hassan M, Ehrsson H, Ljungman P. Aspects concerning busulfan pharmacokinetics and bioavailability. *Leuk Lymphoma*. 1996 Aug; 22(5–6):395–407. [PubMed: 8882952]
2. Ciurea SO, Andersson BS. Busulfan in hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2009 May; 15(5):523–36. [PubMed: 19361744]
3. Andersson BS, Madden T, Tran HT, Hu WW, Blume KG, Chow DS, Champlin RE, Vaughan WP. Acute safety and pharmacokinetics of intravenous busulfan when used with oral busulfan and cyclophosphamide as pretransplantation conditioning therapy: a phase I study. *Biol Blood Marrow Transplant*. 2000; 6(5A):548–54. [PubMed: 11071260]
4. Slattery JT, Sanders JE, Buckner CD, Schaffer RL, Lambert KW, Langer FP, Anasetti C, Bensinger WI, Fisher LD, Appelbaum FR, et al. Graft-rejection and toxicity following bone marrow transplantation in relation to busulfan pharmacokinetics. *Bone Marrow Transplant*. 1995 Jul; 16(1): 31–42. [PubMed: 7581127]
5. Slattery JT, Clift RA, Buckner CD, Radich J, Storer B, Bensinger WI, Soll E, Anasetti C, Bowden R, Bryant E, Chauncey T, Deeg HJ, Doney KC, Flowers M, Gooley T, Hansen JA, Martin PJ, McDonald GB, Nash R, Petersdorf EW, Sanders JE, Schoch G, Stewart P, Storb R, Sullivan KM, Thomas ED, Witherspoon RP, Appelbaum FR. Marrow transplantation for chronic myeloid leukemia: the influence of plasma busulfan levels on the outcome of transplantation. *Blood*. 1997 Apr 15; 89(8):3055–60. [PubMed: 9108427]
6. Grochow LB. Busulfan disposition: the role of therapeutic monitoring in bone marrow transplantation induction regimens. *Semin Oncol*. 1993 Aug; 20(4 Suppl 4):18–25. quiz 26.
7. Dix SP, Wingard JR, Mullins RE, Jerkunica I, Davidson TG, Gilmore CE, York RC, Lin LS, Devine SM, Geller RB, Heffner LT, Hillyer CD, Holland HK, Winton EF, Saral R. Association of busulfan area under the curve with veno-occlusive disease following BMT. *Bone Marrow Transplant*. 1996 Feb; 17(2):225–30. [PubMed: 8640171]
8. McCune JS, Gibbs JP, Slattery JT. Plasma concentration monitoring of busulfan: does it improve clinical outcome? *Clin Pharmacokinet*. 2000 Aug; 39(2):155–65. Review. [PubMed: 10976660]
9. Matthews RH, Emami M, Connaghan DG, Holland HK, Morris LE. Home administration of high-dose oral busulfan in patients undergoing hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2007 Apr; 39(7):397–400. [PubMed: 17322933]
10. Russell JA, Kangaroo SB. Therapeutic drug monitoring of busulfan in transplantation. *Curr Pharm Des*. 2008; 14(20):1936–49. [PubMed: 18691105]

11. Harvey RD, Shea T, Walko C, Krasnow C, Patel L, Serody J, Gabriel D, Comeau T, Lindley C. Intravenous busulfan test dose clearance comparison with first and thirteenth dose systemic exposure in allogeneic bone marrow transplantation patients. *Journal of Clinical Oncology*, 2005 ASCO Annual Meeting Proceedings. 2005; 23(16S June 1 Supplement):6658.
12. Walko CM, Lindley CM, Collins J, Krasnov C, Harvey RD, Gabriel DA, Serody J, Comeau T, Shea TC. 90 hour continuous intravenous busulfan infusion: PK and test dose predictability in allogeneic bone marrow transplantation patients. *Journal of Clinical Oncology*, 2005 ASCO Annual Meeting Proceedings. 2005; 23(16S, Part I of II June 1 Supplement):6652.
13. Walko CM, Shea TC, Rao K, Gabriel D, Serody J, Comeau T, Gilman A, Krasnov C, Lindley CM. Phase I study utilizing an intravenous busulfan test dose to prospectively target and determine the maximum tolerated systemic exposure (MTSE) of a continuous intravenous infusion. *Journal of Clinical Oncology*, 2006 ASCO Annual Meeting Proceedings Part I. 2006; 24(18S June 20 Supplement):16502.
14. Beri R, Chunduri S, Sweiss K, Peace DJ, Mactal-Haaf C, Dobogai LC, Shord S, Quigley JG, Chen YH, Mahmud N, Rondelli D. Reliability of a pretransplant i.v. BU test dose performed 2 weeks before myeloablative FluBu conditioning regimen. *Bone Marrow Transplant*. 2010 Feb; 45(2):249–53. [PubMed: 19543331]
15. Reis EO, Vianna-Jorge R, Suarez-Kurtz G, Silva Lima EL, Almeida Azevedo D. Development of a rapid and specific assay for detection of busulfan in human plasma by high-performance liquid chromatography/electrospray ionization tandem mass spectrometry. *Rapid Commun Mass Spectrom*. 2005; 19:1666–1674. [PubMed: 15912469]

Figure 1.
Typical conc./time and semi-log (log conc./time) plots.

Figure 2. Plot 1
Correlation AUC_{therap} vs AUC_{test}

Table 1

Treatment flow chart

DRUG/DOSE	DAY -4	DAY -3	DAY -2	DAY -1	DAY 0-4
Alemtuzumab (<i>in vivo</i>) 20 mg/day	x	x	x	x	
Fludarabine 40 mg/m ² /day	x	x	x	x	
Busulfan TEST dose 13 mg/m ² (18 min infusion)	x				
Busulfan THERAPY dose 130 mg/m ² /day (180 min infusion)		x	x		
Allogeneic PBSC infusion					x

Table 2

AUC (Therapeutic) vs AUC (Test) data from 3 patients

Predictability of i.v. busulfan 1/10 TEST DOSE			
AUC _{test}	AUC _{therap} (ACTUAL)	AUC _{therap} (PREDICTED)	% error in prediction
447	3367	3330	-1
376	3186	3126	-2
403	3106	3203	3