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Influence of Metronidazole on Oral Busulfan Test Dose

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Keywords

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Background and Objectives

Individualization of busulfan (BU) dosing during pre-transplant conditioning for hematopoietic stem cell transplantation (HSCT) has been performed worldwide for more than a decade. The pharmacokinetic parameters of BU show high variability and studies report that high plasma concentrations (> 900 ng/mL) are associated with sinusoidal obstructive syndrome (SOS), whereas low plasma concentrations (< 600 ng/mL) are associated with graft rejection and/or disease recurrence [1,2]. The high variability in BU pharmacokinetics can result in severe adverse reactions and can compromise the success of transplantation. One alternative used for the individualization of BU therapy is the application of a test dose days before the beginning of conditioning. On the basis of a small test dose of BU administered days before conditioning, drug clearance by the patient is calculated and the ideal dose to achieve the target plasma concentration is defined. The major disadvantage of this technique is that the BU test dose is administered prior to the use of different drugs present in the pre-HSCT conditioning regimen, such as antibiotics, glucocorticoids and anticonvulsants, among others [3-7].

Busulfan is metabolized by conjugation with glutathione which is mediated by glutathione-S-transferase, followed by oxidation catalyzed by CYP3A4 [2,4,5,8,9]. The main metabolites of BU are 3-hydroxysulfolane and tetrahydrothiophene 1-oxide, which do not significantly contribute to the efficacy or toxicity of the drug [10-12].

Several drugs that may have an impact on the clearance of BU have been described in the literature, including the antibiotic metronidazole which is used during pre-HSCT conditioning to prevent infections caused by *Clostridium difficile* and/or to avoid graft-versus-host disease [12-15]. We report a clear case report of drug-drug interaction between BU and oral metronidazole, in which the patient received two test doses of BU, one in combination with metronidazole and one without this drug.

Case Report

The study was approved by the Research Ethics Committee of the University Hospital of the School of Medicine of Ribeirao Preto (HCFMRP-USP) and the patient was only submitted to investigation after he had given free informed consent.

A 30-year-old male patient (73 kg, BMI: 24.9 kg/m^2) with chronic myeloid leukemia was submitted to HSCT at the Bone Marrow Transplant Unit of HCFMRP-USP. On the third day of treatment with metronidazole (400 mg/8h), the patient received an oral test dose of BU (0.25 mg/kg) (Myleran®, GlaxoSmithKline). Serial blood samples (volumes of 1 mL) were collected with heparinized syringes at times 0, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 180, 240, 300, and 360 min after administration of the BU test dose. The plasma samples were immediately analyzed by LC-MS/MS [16] and the pharmacokinetic parameters were determined with the WiNonLin 5.2 software using a mono-compartmental model.

The clearance calculated based on the administration of the BU test dose combined with metronidazole was 10.3 L/h. The dose suggested to achieve C^{ss} within the target range of 600-900 ng/mL according to the equation dose/τ = Cl/f .C^{ss}, where τ corresponds to the 6-h dose interval, would be 37 to 55.6 mg/kg/6 h for 4 days. However, due to problems related to bone marrow donation, the patient did not start the pre-HSCT conditioning. Two months later, the same patient was rehospitalized for HSCT. A new test dose of BU (0.25 mg/kg) was administered; however, on this occasion, the test was performed before the use of metronidazole. On this second occasion, the apparent clearance value of the patient was 16.6 L/h and the ideal dose suggested was 60-90 mg/kg/6 h for 4 days. The patient was conditioned with an oral BU dose of 72 mg/kg administered at intervals of 6 h for 4 days.

After administration of the fifth BU dose during the conditioning regimen, serial blood samples were collected during the 6-h dose interval and immediately analyzed to determine whether the C^{ss} of BU was within the target range of 600-900 ng/mL. The AUC calculated for the 6-h dose interval using a non-compartmental model (steady state) was 4,516.5 ng.h/mL, corresponding to C^{ss} of 752.7 ng/mL.

Figure 1 shows the decay curves of plasma BU concentrations over time after administration of the test doses and of the fifth dose.

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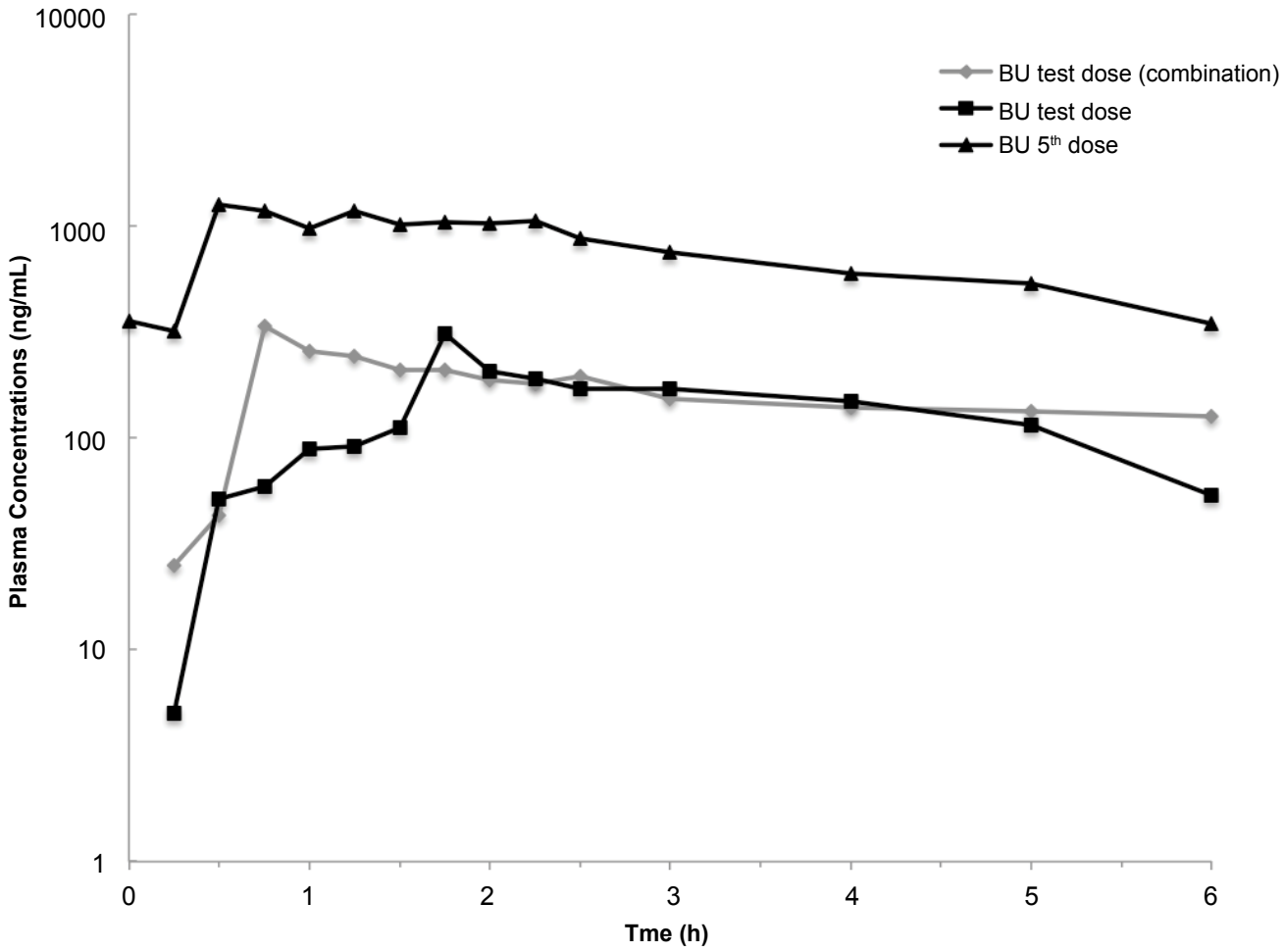

Figure 1: Plasma busulfan (BU) concentration versus time during the test dose (combined or not with metronidazole) and during the 5th BU dose.

Discussion

The individualization of oral or intravenous BU therapy is a routine procedure at most HSCT centers. Some centers perform therapeutic monitoring on the first day of conditioning, whereas others use a test dose [3,4,17,18]. We found only two studies in the literature reporting BU - metronidazole interaction, one using oral administration and the other intravenous administration [6,12]. Nilsson et al. [12] compared the plasma concentrations of BU measured immediately before each dose (trough levels) between three groups of patients: group A received oral BU combined with metronidazole (400 mg/8 h), group B was treated with oral BU without metronidazole for 2 days and received the combination (400 mg/8 h) on the subsequent 2 days, and group C was only treated with BU and did not receive metronidazole. The authors observed a significant difference in plasma BU concentrations between groups A and C, an increase in plasma BU concentrations in group B when the patients began treatment with metronidazole, and marked hepatic alterations in patients receiving the combination. Gulbis et al. [6] reported the case of a 7-year-old child who received an intravenous test dose of BU (0.5 mg/kg) 7 days before HSTC and began treatment with oral metronidazole 6 days before HSTC. The authors observed a 46% reduction in clearance after the beginning of metronidazole treatment, which led to the discontinuation of the last BU dose in an attempt to prevent the occurrence of SOS. As a consequence, the patient did not develop SOS, but relapsed 3 months after HSCT.

The present study reports the case of an adult patient with chronic myeloid leukemia who received two oral test doses of BU, one combined with metronidazole (400 mg/8 h) and the other without the drug. A reduction in clearance of 38% was observed for the combination of oral BU and metronidazole. If this dosage had been continued, the patient would probably have presented low plasma concentrations of BU, a fact that could have resulted in recurrence of the disease and/or graft rejection. Since the same patient was enrolled, the possibility that this altered clearance was due to genetic changes, the influence of age, or even the influence of the underlying condition could be ruled out. Therefore, the new oral test dose of BU was crucial to the success of the treatment. Plasma BU concentrations were evaluated after the fifth BU dose and the results showed C^{ss} values within the target range. The patient was in clinical remission in the end of this study.

The mechanism whereby metronidazole interferes with the elimination of BU is still unclear and, although BU is metabolized through conjugation with glutathione, most drugs that interfere with the metabolism of BU are inducers or inhibitors of CYP enzymes that are involved in the elimination of intermediate metabolites of BU [19,20]. *In vitro* data have shown that metronidazole can inhibit CYP3A [21], however, recent *in vivo* data using high doses of metronidazole are contrary to these results [22,23]. The hypothesis of BU as a substrate of P-gp and of metronidazole as an inhibitor of P-gp was considered, but rapidly discarded since previous studies reported the influence of metronidazole on the metabolism of BU during both intravenous and oral administration [6,12]. Another possible and strong hypothesis is related to the inhibition of CYP2C9. Although the participation of CYP2C9 in the metabolism of BU has never been reported in the literature, according to the recent study by Uppunguduri et al. [24], patients genotyped as CYP2C9*2 and *3 show higher metabolic ratios (BU/3-hydroxysulfolane) than patients who did not carry these allelic variant, suggesting a relationship between CYP2C9 activity and BU metabolism. These results clarify the influence of metronidazole on the pharmacokinetics and metabolism of BU, since the former is a weak inhibitor of CYP2C9 in *in vivo* studies [25].

The main objective of the present case report was to alert HSCT centers that perform BU dose adjustments based on a test dose to observe whether metronidazole is administered during the test dose and/or during the conditioning period with BU in order to prevent a wrong dose prediction as a consequence of drug-drug interaction.

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