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Formation Rate–Limited Pharmacokinetics of Biologically Active Epoxy Transformers of Prodrug Treosulfan

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ABSTRACT

A prodrug treosulfan (TREO) is being evaluated in clinical trials as a myeloablative agent before hematopoietic stem cell transplantation. The active derivatives of TREO, monoepoxide (EBDM), and diepoxide (DEB), are formed in a pH-dependent nonenzymatic reaction. The aim of the study was to investigate pharmacokinetics of the TREO epoxy transformers in a rabbit model and explain the causes of low plasma concentrations of EBDM and DEB observed in patients receiving high-dose TREO before hematopoietic stem cell transplantation. New Zealand white rabbits (n = 5 per cohort) received an intravenous infusion of TREO (group I), injection of DEB (group II), and injection of a solution containing EBDM (group III). When EBDM and DEB were administered to the rabbits, they underwent a very rapid elimination (half-life 0.069 and 0.046 h) associated with a high systemic clearance (10.0 and 14.0 L h^{-1} kg^{-1}). After administration of TREO, the $t_{1/2}$ of EBDM was statistically equal to the $t_{1/2}$ of the prodrug (1.6 h). To conclude, after administration of TREO, its epoxy transformers demonstrate a formation-limited elimination. Then EBDM and DEB have the same elimination half-life as TREO, but the levels of EBDM and DEB in the body, including plasma, are much lower than TREO on account of their inherently high clearance.

Introduction

In the recent years, an anticancer drug treosulfan (TREO), a structural analogue of busulfan, has been increasingly applied to conditioning before hematopoietic stem cell transplantation (HSCT) in pediatric and adult patients. A main rationale for that approach lies in the clinical experience that high-dose TREO combines an efficient myeloablation and lower organ toxicity, especially hepatotoxicity, compared with busulfan.[1-9] Currently, a multicenter randomized phase III clinical trial is ongoing to investigate TREO/fludarabine versus busulfan/fludarabine conditioning regimens before allogeneic HSCT in adult patients with acute myeloid leukemia and myelodysplastic syndromes.[10] TREO is a prodrug which at pH >5 undergoes a 2-step nonenzymatic activation to (2S,3S)-1,2-epoxy-3,4-butanediol 4-methanesulfonate (S,S-EBDM) and (2S,3S)-1,2:3,4-diepoxybutane (S,S-DEB). These derivatives alkylate the DNA, which results in cytotoxic effects underlying anticancer and/or myeloablative action observed after TREO administration (Fig. 1).[7,11,12] So far, several pharmacokinetic studies of intravenous TREO have been conducted in adult and pediatric patients.[13-20] The drug changes in plasma were best described by a 2-compartment model with an elimination half-life ($t_{1/2\ \beta}$) close to 2 h, no matter of the dose applied (8-47 g/m^2).[13-19] A renal clearance of unchanged TREO was designated to be about 25% of its total clearance (Cl_{tot}), which accords with a urine excretion of 15%-40% of the prodrug dose in the unchanged form.[13-18] In a phase I clinical trial conducted in adult patients, TREO exhibited a

Abbreviations used: $AUC_{0\rightarrow\infty}$, area under the plasma drug concentration–time profile from zero to infinity; Cl_{tot}, total clearance; C_{max}, maximal concentration of a drug in plasma; (±)-DEB, (±)-1,2:3,4-diepoxybutane; HSCT, hematopoietic stem cell transplantation; LOQ, limit of quantitation; NA, not applicable; RID, refractive index detector; R.S.D., relative standard deviation; S,S-DEB, (2S,3S)-1,2:3,4-diepoxybutane; S,S-EBDM, (2S,3S)-1,2-epoxybutane-3,4-diol-4-methanesulfonate; $t_{1/2\ \alpha}$, half-life of a drug distribution phase; $t_{1/2\ \beta}$, half-life of a drug elimination phase (elimination half-life); TREO, treosulfan; V_c, volume of a central compartment; V_{ss}, volume of distribution at steady state; V_t, volume of a tissue compartment.

Conflicts of interest: The authors declare no conflict of interests.

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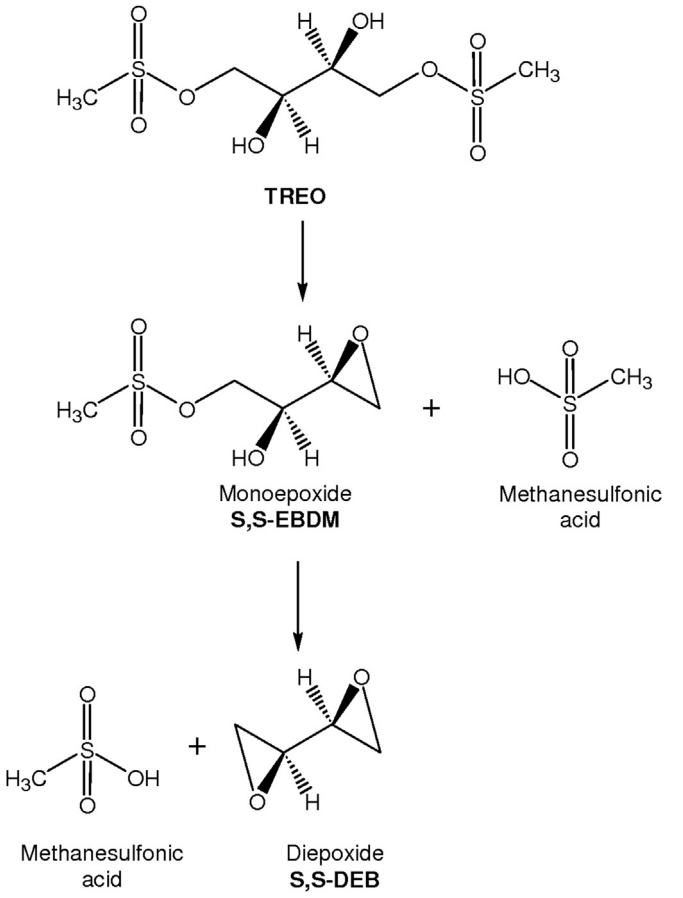

Figure 1. Nonenzymatic conversion of TREO to biologically active epoxides.

linear change of the area under the plasma drug concentration–time profile ($AUC_{0\rightarrow\infty}$) in a wide range of doses, 20-47 g/m^2.[13] The knowledge of pharmacokinetics of S,S-EBDM and (2S,3S)-1,2:3,4-diepoxybutane is still very scarce. The current literature presents only one report describing pharmacokinetic parameters of S,S-EBDM in 16 children undergoing TREO-based conditioning before HSCT. In those patients, the $t_{1/2\ \beta}$ of S,S-EBDM was similar to that of the parent drug, although the measured plasma concentrations of the monoepoxide were 2 orders of magnitude lower.[18] No detectable plasma S,S-DEB levels were found in the patients' plasma samples after TREO admininstration.[21] In a rat model, a racemate of 1,2:3,4-diepoxybutane ((±)-DEB) administered intravenously at the dose of 45 mg/kg body weight (b.w.) demonstrated a fast disposition with a $t_{1/2\ \beta}$ equal to 14 min.[22] The same compound was found to undergo enzyme-mediated utilization by hydrolysis and conjugation to glutathione in the presence of liver and lung microsomes *in vitro*.[23,24] When the 3 stereoisomers of 1,2:3,4-diepoxybutane (S,S, R,R, and meso) were incubated with a mixture of reduced glutathione and glutathione S-transferase, the catalytic efficiency of the conjugates formation was highest for S,S-DEB and lowest for the meso-isomer; however, the difference was <3-fold.[25] Recently, it has been demonstrated that at pH 7.4 and 37°C, the nonenzymatic hydrolytic decomposition of S,S-EBDM and S,S-DEB in the presence of the human plasma electrolytes proceeds so slow that cannot account for their surprisingly low levels observed in the patients receiving TREO.[26] In this article, pharmacokinetics of S,S-EBDM and S,S-DEB after intravenous infusion of TREO and injection of the preformed epoxides was studied in a rabbit model for the first time.

Materials and Methods

Caution: Treosulfan and 1,2:3,4-diepoxybutane are hazardous and should be handled using the appropriate safety precautions.

Drugs and Chemicals

TREO in the form of a powder for solution for injection or infusion was kindly supplied by medac GmbH (Wedel, Germany). (±)-DEB, acetaminophen, 2,2′-dinitrobiphenyl, and sodium acetate were purchased from Sigma-Aldrich (St. Louis, MO). 3-Nitrobenzenesulfonic acid was obtained from TCI Europe NV (Boerenveldsweg, Belgium). Sodium hydroxide, acetic acid, and citric acid were all of analytical grade, and acetonitrile and dichloromethane were HPLC gradient grade. These chemicals were purchased from commercial suppliers. Demineralized water with a conductivity of 0.1 μS cm^{-1}was prepared in a deionizer Simplicity UV (Millipore, Billerica, MA) and filtered through a 0.45-μm cellulose membrane filter (Sartorius, Goettingen, Germany) before use. Lyophilized rabbit plasma for reconstitution in water was purchased from Biomed (Cracow, Poland). Heparin (Heparinum WZF) was obtained from WZF Polfa (Warsaw, Poland) and Ondansetron (Ondansetron Kabi) from Fresenius Kabi (Warsaw, Poland).

Animals

The studies were performed in 15 adult New Zealand white rabbits of both sexes (3.4-5.2 kg, median 4.2 kg) on approval of the Local Ethics Committee for Experimental on Animals in Poznan, Poland. The advantage of the use of rabbits, compared with rodents, was a possibility to administer TREO as an intravenous infusion, which is a typical route of the drug administration applied in the patients undergoing HSCT, obtain a full concentration–time profile from each individual animal and, consequently, limit the number of the used specimens. The animals were kept singly in standard cages under controlled temperature of 21 ± 1°C and 12 h light/dark cycle and were provided with 125 g of the commercial pelleted feed per day and tap water *ad libitum*. Twelve hours before the drugs administration, the animals were fasted. All animal procedures were conducted in accordance with the European Community guidelines as accepted principles for the use of experimental animals, and every possible effort was made to minimize animal suffering.

Administration of TREO and Its Epoxy Transformers and the Blood Samples Collection

To study pharmacokinetics of TREO and its epoxy transformers, the animals were randomly divided into 3 equinumerous groups (5 rabbits per group) which received TREO (group I), commercially available (±)-DEB (group II), and a mixture of TREO S,S-EBDM and S,S-DEB (group III). Before the experiment, the rabbits were weighed and placed into restraining cages. The skin over the auricular artery was cleaned with alcohol, and a catheter for blood collection was inserted into the central auricular artery and fixed to the skin with a tape. On the second ear, another catheter was placed in the marginal vein to administer the medicines. Both the auricular catheter systems were flushed with 1000 IU of heparin in saline. Ten minutes before the drugs administration, the rabbits received 2 mg of antiemetic ondansetron as an intravenous injection.

The specimens from the group I (weight 4.2-4.4 kg, median 4.2 kg) received 340 mg/kg b.w. of TREO (1.22 mmol/kg b.w.) as a 15-min infusion of a freshly prepared 40 mg mL^{-1} solution of TREO