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Effect of Temperature on the Kinetics of the Activation of Treosulfan and Hydrolytic Decomposition of Its Active Epoxy Derivatives

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ABSTRACT

Treosulfan (TREO) is a prodrug applied in the treatment of ovarian cancer and a myeloablative conditioning prior to stem cell transplantation. A sequential activation of TREO to intermediate monoepoxide (S,S-EBDM) and then to (2S,3S)-1,2:3,4-diepoxybutane (S,S-DEB) involves a nonenzymatic intramolecular nucleophilic substitution. The aim of this study is to determine the effect of temperature on the rate constants (k) for the activation of TREO and the hydrolysis of its epoxy derivatives in a phosphate buffer of pH 7.4 at an ionic strength of 0.16-0.17 M. Over the tested temperature range, the ln of k changed linearly with a reciprocal of absolute temperature. The mean activation energy (E_a) values for the TREO → S,S-EBDM and S,S-EBDM → S,S-DEB reactions were close to each other (122 and 118 kJ/mol, respectively). In opposition, the E_a for the hydrolysis of S,S-EBDM and S,S-DEB differed significantly (140 and 80 kJ/mol, respectively), which indicates that the structure of S,S-EBDM hampers a nucleophilic attack of water on the epoxide ring. The obtained results show that a temperature change by 1°C, from 36.5°C to 37.5°C, entails a 17% increase in the k of TREO decay, which might lead to an increased TREO clearance *in vivo*.

Introduction

Treosulfan (TREO), (2S,3S)-1,2:3,4-butanetetraol 1,4-bismethanesulfonate, belongs to alkylating agents. TREO is registered in several European countries for the treatment of advanced ovarian cancer, either in intravenous regimens (single dose of 3-8 g/m^2 given every 1-4 weeks) or in oral ones (1 or 1.5 g daily for 1-4 weeks followed by 2-4 weeks off therapy).[1–3] Higher intravenous doses of the drug, that is, 10-14 g/m^2 given for 3 consecutive days, have been applied successfully in clinical trial setting to a myeloablative conditioning prior to hematopoietic stem cell transplantation. A combination of high-dose TREO with fludarabine is currently being investigated in Europe, the United States, and Israel as a reduced toxicity conditioning that can be offered to adult and pediatric patients ineligible for standard regimens.[4–8] TREO is a synthetic prodrug which at pH above 5 undergoes a sequential activation to the 2 biologically active epoxy derivatives, the intermediate monoepoxide, (2S,3S)-1,2-epoxy-3,4-butanediol 4-methanesulfonate (S,S-EBDM), and finally (2S,3S)-1,2:3,4-diepoxybutane (S,S-DEB), as shown in Figure 1.[9-12] Only about 25% of TREO is excreted in the unchanged form in urine, and the conversion to S,S-EBDM seems to be a major route of TREO elimination from the body.[12-16] Therefore, the changes in the kinetics of the above reaction are anticipated to affect pharmacokinetics of the prodrug.[12] From a mechanical point of view, the TREO activation is established to involve an intramolecular nucleophilic substitution of the methanesulfonate moiety by the deprotonated β-hydroxyl group. The rate of this process increases with pH because a more efficient deprotonation of the hydroxyl groups occurs.[17-21] However, the influence of temperature on the TREO transformation has not been investigated so far. In clinical conditions, the body temperature of patients undergoing conditioning prior to hematopoietic stem cell transplantation usually ranges from 36.5°C to 37.5°C. However, in high-risk patients who have experienced prior intensive treatments, higher body temperatures can be observed. Moreover, during TREO-based conditioning a neutropenia starts even on the second day of the regimen; therefore, next 1 or 2 doses of TREO may be given to the patient with fever.[22] Incidents of pyrexia can also happen during cyclic

Abbreviations used: A, pre-exponential factor; ΔE_a, standard error of the activation energy; E_a, activation energy; k, reaction rate constant; k_1, rate constant for the reaction TREO → S,S-EBDM; k_2, rate constant for the reaction S,S-EBDM → S,S-DEB; k_D, rate constant for the hydrolysis of S,S-DEB; k_M, rate constant for the hydrolysis of S,S-EBDM; r, correlation coefficient; R^2, global coefficient of determination; SE, standard error; S,S-DEB, (2S,3S)-1,2:3,4-diepoxybutane; S,S-EBDM, (2S,3S)-1,2-epoxybutane-3,4-diol-4-methanesulfonate; $t_{1/2}$, reaction half-life; TREO, treosulfan.

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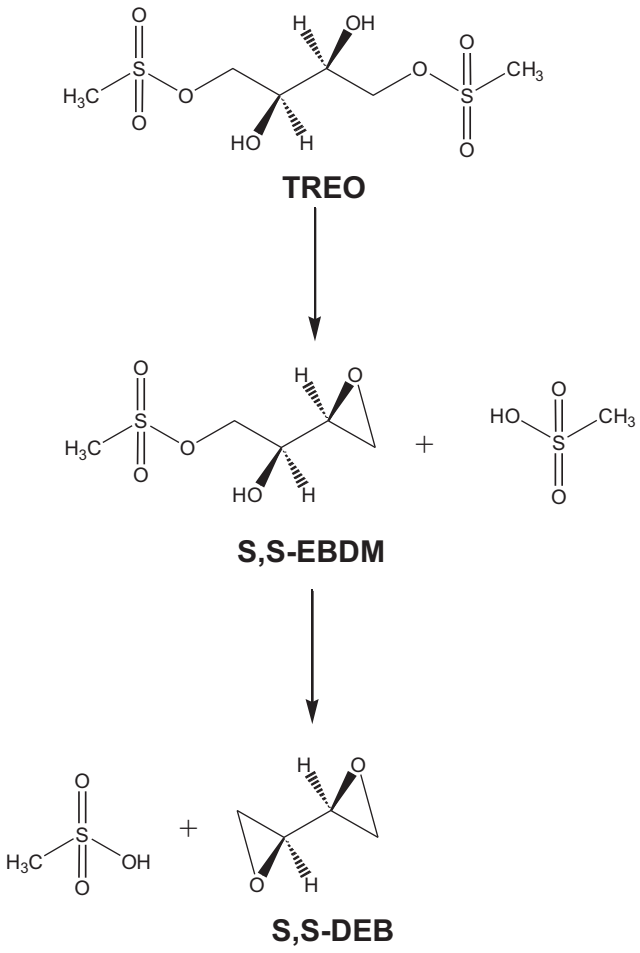

Figure 1. TREO conversion to biologically active epoxides.

chemotherapies of ovarian cancer with TREO, especially in oral regimens when the drug is taken every day continuously for 1-4 weeks.[2] Therefore, for the first time, in the present paper we have investigated the effect of temperature on the sequential conversion of TREO to S,S-EBDM and S,S-DEB and also on the hydrolysis of the both epoxy transformers at physiological pH 7.4 and an ionic strength of 0.16-0.17 M. The activation energies (E_a) and pre-exponential factors for the above reactions were calculated using the Arrhenius equation and discussed. The obtained results provide a new insight into rather scarce data on the kinetics of epoxide reactions. The increase in the rate of the epoxy transformation of TREO with temperature turned out to be so huge that the body temperature change of even 1°C may be of clinical importance for the pharmacokinetics of TREO.

Materials and Methods

Caution

TREO is hazardous and should be handled using the appropriate safety precautions.

Chemicals and Reagents

TREO was kindly donated by Medac GmbH (Wedel, Germany). Acetaminophen and sodium acetate were purchased from Sigma-Aldrich (Steinheim, Germany). Sodium hydroxide, 0.1 M sodium hydroxide volumetric solution, disodium hydrogen phosphate, potassium dihydrogen phosphate, glacial acetic acid, and citric acid, all analytical grade, were obtained from P.O.Ch. (Gliwice, Poland). Acetonitrile, HPLC gradient grade, was purchased from Merck KGaA (Darmstadt, Germany). Certified pH calibration buffers (DuraCal pH 4.01, 7.00, and 10.01) were from Hamilton (Bonaduz, Switzerland). Demineralized water with a resistance of not less than 18 MΩ·cm was prepared in a deionizer Simplicity UV (Millipore, Billerica, MA) and filtered through a 0.45-μm cellulose filter prior to use. As S,S-EBDM and S,S-DEB are not commercially available, a stock solution of TREO, S,S-EBDM, and S,S-DEB for a quantitative HPLC analysis was obtained by titrating the aqueous solution of TREO with an equimolar amount of NaOH, as described previously.[23]

Stock Solution of TREO

A stock solution of 0.15 M TREO for kinetic studies was prepared by dissolving 0.4174 g of the compound in water and diluting to a final volume of 10 mL with water in a volumetric flask.

Preparation of the pH 7.4 Phosphate Buffer

To prepare the pH 7.4 phosphate buffer, 0.716 g of sodium hydrogen phosphate and 0.150 g of potassium dihydrogen phosphate were dissolved in 95 mL of water. The resulting solution was heated to the target temperature of 40°C, 50°C, 60°C, 70°C, or 80°C and adjusted to pH 7.40 with 1 M NaOH. Thereafter, the solution was cooled to room temperature and diluted to a final volume of 100 mL with water. The ionic strength of the obtained phosphate buffer at 40°C-80°C was in the range 0.16-0.17 M.

Activation of TREO at Different Temperatures

Stoppered glass vials containing 9.967 mL of the pH 7.4 phosphate buffer ($n = 3$) were placed in a water bath. Once the target temperature (40°C-80°C) was reached, the buffer was spiked with 33.3 μL of the 0.15 M stock solution of TREO to obtain the initial concentration of the prodrug of 0.5 mM. When the transformation of TREO to S,S-EBDM and S,S-DEB was proceeding, 250 μL samples of the studied solution were collected with an HPLC glass syringe (Hamilton, Reno, NV) and immediately transferred into 1.5 mL vials which were placed in ice and contained 25 μL of 0.3 M citric acid solution to prevent the further, artificial transformation of TREO and S,S-EBDM. The concentrations of TREO, S,S-EBDM, and S,S-DEB in the collected samples were determined with a validated HPLC method with refractive index detection as described elsewhere.[23]

Kinetic Calculations

Based on the changes in concentrations of TREO and its epoxy transformers in the pH 7.4 phosphate buffer, the rate constants for the TREO transformation were estimated in Prism 6.01 (GraphPad Software Inc., San Diego, CA) using the previously developed kinetic model.[12] This model included 2 first-order reaction of TREO → S,S-EBDM and S,S-EBDM → S,S-DEB, and 2 parallel pseudo-first-order reactions of hydrolytic decay of S,S-EBDM and S,S-DEB (Fig. 2). Validation of the model was performed in each single experiment according to the guidelines for evaluation of nonlinear models.[12] The half-lives ($t_{1/2}$) for the studied reactions were calculated as 0.693 dived by the relevant rate constant. The natural logarithms of the obtained mean rate constants were plotted against a reciprocal of absolute temperature and the parameters of the obtained lines, that is, the slope (a), standard error of the slope (S_a), intercept (b), standard error of the intercept (S_b), and correlation coefficient (r), were determined using a regression tool in Excel 2010 (Microsoft Corporation, USA). Thereafter, the Arrhenius