Drug Metabolism and Pharmacokinetics 31 (2016) 356–362

Contents lists available at ScienceDirect

Drug Metabolism and Pharmacokinetics

journal homepage: http://www.journals.elsevier.com/drug-metabolism-and-pharmacokinetics

Regular Article

# Ocular disposition of treosulfan and its active epoxy-transformers following intravenous administration in rabbits

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ARTICLE INFO

*Article history:*
Received 21 April 2016
Received in revised form
24 June 2016
Accepted 6 July 2016
Available online 14 July 2016

*Keywords:*
Blood – aqueous barrier
Permeability
Uveal melanoma
Pharmacokinetics
Prodrug
Epoxides
HPLC

ABSTRACT

Treosulfan (TREO) has an established position in chemotherapy of advanced ovarian cancer but has been also applied in uveal melanoma patients. Moreover, it is used as an orphan drug for a myeloablative conditioning prior to stem cell transplantation. In this paper, biodistribution of prodrug TREO and its active monoepoxide (S,S-EBDM) and diepoxide (S,S-DEB) into aqueous humor of the eye was studied for the first time. For that purpose, alone TREO and the mixture of TREO, S,S-EBDM and S,S-DEB were administered intravenously to New Zealand White rabbits. The three analytes were determined in plasma and aqueous humor by validated HPLC methods and pharmacokinetic calculations were performed in WinNonlin. After the infusion of TREO, the aqueous humor/plasma $C_{max}$ ratio and area under the curve ratio amounted 0.04 and 0.10 for TREO, and 1.1 and 2.2 for S,S-EBDM, respectively. Following the bolus injection of the mixture of the prodrug and its epoxides, the aqueous humor/plasma $C_{max}$ ratios for TREO, S,S-EBDM and S,S-DEB were 0.05, 0.66, and 4.0, respectively. The presented results indicate a poor penetration of TREO into the eye, which may impair systemic treatment of ocular tumors but is beneficial in terms of a lack of clinically relevant ophthalmic adverse effects.



## 1. Introduction

Treosulfan (TREO) is registered in some European countries for treatment of advanced ovarian carcinoma with the usage of doses up to 8 g/m$^2$. In preclinical studies, the drug also demonstrated anticancer activity towards variety of tumors, even those resistant to other cytotoxic agents [1–6]. Application of higher doses of TREO (10–14 g/m$^2$ for three consecutive days) provides a strong myeloablative action but still relatively low organ toxicity. Therefore, currently, TREO is evaluated in phase II and III clinical trials as a myeloablative component of conditioning regimens prior to hematopoietic stem cell transplantation (HSCT) [7–11]. Considering the mechanism of action, TREO is a prodrug of two alkylating transformers, (2S,3S)-1,2-epoxy-3,4-butanediol 4-methanesulfonate (S,S-EBDM) and (2S,3S)-1,2:3,4-diepoxybutane (S,S-DEB), which are capable of alkylating the DNA. The active epoxides are formed at pH > 5 via a two-step nonenzymatic reaction of intramolecular nucleophilic substitution (Fig. 1) [12,13].

The knowledge of biodistribution of drugs into a specific tissue provides better understanding of their regional pharmacological activity as well as potential adverse effects. In a rat model, TREO and S,S-EBDM weakly penetrated across the blood – brain barrier (BBB), which may explain a small clinical efficacy of TREO against malignant glioma and, on the other hand, relatively small neurotoxicity of the drug in HSCT patients [14]. Another specific barrier in the body is the blood – ocular barrier, which protects the eye against systemically administered drugs. However, this barrier may also obstruct systemic treatment of ocular diseases, for instance systemic chemotherapy applied in common intraocular primary carcinoma, that is lymphoma and metastatic melanoma in adult patients and advanced retinoblastoma in children [15–21]. So far, TREO alone or in combination with other anticancer drugs, *e.g.*, gemcitabine, cytarabine, and paclitaxel, was reported to be cytotoxic towards primary uveal melanoma tumors in *ex vivo* testing, at concentrations corresponding to those observed in the patients' plasma [6,22]. In metastatic uveal melanoma patients with poor prognosis a partial remission or stabilization of the disease was sometimes noted after TREO-based chemotherapy [23–26]. In

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http://dx.doi.org/10.1016/j.dmpk.2016.07.001
1347-4367/

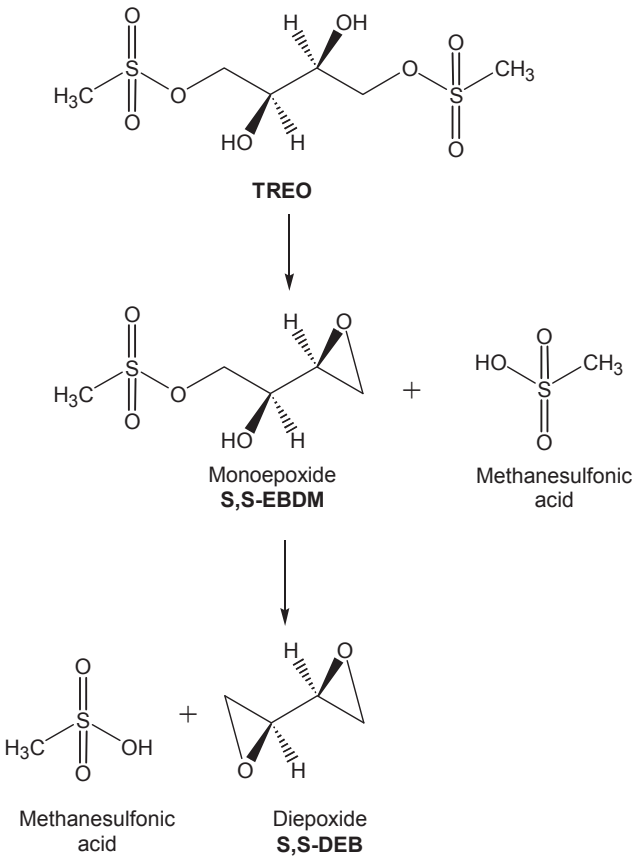


**Fig. 1.** Conversion of TREO to the biologically active epoxy-derivatives.

addition, megachemotherapy with high-dose TREO followed by autologous HSCT provided complete remissions in relapsed high-grade systemic lymphoma [27]. On the other hand, systemic anticancer therapies and HSCT procedure are associated with ocular complications in children and adults [28–30]. In view of the above facts, data on intraocular penetration of TREO would be useful in prediction and assessment of its potential anticancer activity, for example against ocular lymphoma and melanoma, or toxicity to the eye tissues. Therefore, the results of the *in vivo* disposition of TREO and its active epoxy-transformers, S,S-EBDM and S,S-DEB, into aqueous humor of the rabbit eye are presented in this paper.

## 2. Material and methods

### 2.1. Drugs and chemicals

A certificate standard of TREO was kindly supplied by medac GmbH (Wedel, Germany). Acetaminophen, 2,2′-dinitrobiphenyl and sodium acetate were obtained from Sigma–Aldrich (St. Louis, MO, USA). 3-Nitrobenzenesulfonic acid was purchased from TCI Europe NV (Boerenveldsweg, Belgium). 0.1 M Sodium hydroxide volumetric solution, glacial acetic acid and citric acid, all analytical grade, were obtained from P.O.Ch. (Gliwice, Poland). Acetonitrile (Merck, Darmstadt, Germany) and dichloromethane (P.O.Ch., Gliwice, Poland) were HPLC grade. Demineralized water with a resistance of 18 MΩ cm was prepared in a deionizer Simplicity UV (Millipore, Billerica, MA, USA) and filtered through a 0.45 μm cellulose membrane filter (Sartorius, Göttingen, Germany) prior to use. Lyophilized rabbit plasma for reconstitution in water was obtained from Biomed (Cracow, Poland). Xylasine (Xylavet 2%) was purchased from ScanVet (Gniezno, Poland), and ketamine (Bioketan) from Vetoquinol Biowet (Gorzów Wlkp., Poland).

### 2.2. Animals

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. The experiments have been approved by the local ethics committee for experiments on animals. Ten New Zealand White rabbits of both sexes (3.4–5.2 kg, median 4.2 kg) 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 drug administration the animals were fasted. For the study, the rabbits were randomly assigned consecutive numbers and divided into two groups, A (rabbits No. 1–5) and B (No. 6–10).

### 2.3. Administration of TREO and its epoxy-transformers and the samples collection

The group A received 340 mg/kg b.w. of TREO as a 15-min intravenous infusion. To the group B rabbits, a combination of TREO (28.0 ± 1.4 mg/kg b.w), S,S-EBDM (16.0 ± 0.7 mg/kg b.w.) and S,S-DEB (8.2 ± 0.4 mg/kg b.w.) was given as an intravenous bolus injection of a solution containing approximately equimolar amounts of the three compounds, as described previously [31]. The samples of 3 ml of blood were collected from a central auricular artery catheter at 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, and 6 h after the end of the TREO infusion (group A), and at 0, 2, 4, 8, 12, 16, 20, 30, 60, and 90 min after the intravenous bolus of TREO, S,S-EBDM and S,S-DEB (group B), followed by restoration with equal volume of saline. At the same time intervals, the aqueous humor (about 300 μl) was withdrawn from an anterior chamber of the eye using an insulin syringe. For that purpose, the animals were anestetized 15 min in advance by intramuscular administration of 10 mg/kg b.w. of xylasine and 50 mg/kg b.w. of ketamine. In each rabbit, two samples of aqueous humor were obtained at adjacent time-points, each one from one eye. The collected samples were immediately acidified with 1 M citric acid solution (50 μl per 1 ml of the blood or aqueous humor) to avoid artificial activation of TREO.

### 2.4. HPLC analysis

Concentrations of TREO, S,S-EBDM and S,S-DEB in the rabbit plasma were determined using validated HPLC methods as described elsewhere [31]. Briefly, for determination of TREO, 315 μl of plasma was transferred to Amicon Ultra-0.5 ml vials (cut-off 30 kDa), spiked with 30 μl of water and 30 μl of a solution of acetaminophen (internal standard), and centrifuged. The obtained filtrate was injected into the HPLC system with refractive index detector (RID). To designate the S,S-EBDM and S,S-DEB concentrations, 525 μl of plasma was spiked with 50 μl of water and then extracted with 5 ml of a mixture of dichloromethane and acetonitrile containing 2,2′-dinitrobiphenyl (internal standard). The epoxides were derivatized with 3-nitrobenzenesulfonic acid and the obtained products were subjected to the HPLC-UV analysis. Calibration standards and quality control samples were prepared in the same manner as the studied samples, except that blank rabbit plasma was spiked with the appropriate standard solutions of the analytes. The limits of quantitation for TREO, S,S-EBDM, and S,S-DEB were 10, 0.8, and 1.1 μM, respectively. The calculation of the TREO, S,S-EBDM, and S,S-DEB concentrations in plasma was based on the equations for the prepared calibration curves. To designate