PHARMACOKINETICS, PHARMACODYNAMICS AND DRUG METABOLISM

Preclinical Pharmacokinetic Evaluation of Resveratrol Trimethyl Ether in Sprague-Dawley Rats: the Impacts of Aqueous Solubility, Dose Escalation, Food and Repeated Dosing on Oral Bioavailability

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ABSTRACT: Resveratrol trimethyl ether (*trans*-3,5,4'-trimethoxystilbene, RTE) is a naturally occurring and pharmacologically active resveratrol derivative. To evaluate its suitability as a drug candidate, a pharmacokinetic study was carried out in Sprague-Dawley rats with the emphasis to identify the impact of aqueous solubility, dose escalation, food, and repeated dosing on its oral bioavailability. Upon single intravenous administration (5 mg/kg), RTE displayed moderate clearance (35.5 ± 5.3 mL/min/kg) and a fairly long terminal elimination half-life (511 ± 136 min); dose escalation (5–20 mg/kg) did not cause nonlinear pharmacokinetics. When given orally in suspension (60 mg/kg), RTE was poorly absorbed with negligible bioavailability (< 1.5%), fasting further decreased its bioavailability (<1%). However, when administered in a solution formulated with randomly methylated-β-cyclodextrin (15 mg/kg), RTE was rapidly absorbed with good bioavailability (46.5 ± 4.8%). Dose escalation resulted in increased bioavailability (64.6 ± 8.0%) at the dose of 60 mg/kg. Repeated RTE dosing (7 daily oral doses) did not alter the clearance, terminal elimination half-life and bioavailability. In summary, the aqueous solubility of RTE was a barrier to oral absorption; repeated RTE administrations did not alter its pharmacokinetic profiles; as RTE possessed appropriate pharmacokinetic profiles, further investigation on RTE as a drug candidate is warranted. © 2011 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:4491–4500, 2011

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INTRODUCTION

Resveratrol (*trans*-3,5,4'-trihydroxystilbene Fig. 1 I) is a polyphenonic compound present in diets such as grapevine, red wine, cranberry, blueberry, bilberry and peanut.[1] It attracted substantial interests in biomedical research during the past 15 years.[1] Its beneficial biological activities, including anti-ageing, anti-cancer (both preventive and therapeutic), anti-diabetic, anti-inflammatory, anti-obesity, anti-oxidation, cardio-protection and neuro-protection have been extensively reported.[1] However, due to its inferior pharmacokinetics, which can be characterized by short half-life,[2,3] extensive phase II metabolism (glucuronide or sulphate conjugation),[4,5] and low bioavailability,[5,6] resveratrol does not appear to be an appropriate drug candidate for further pharmaceutical development. It is therefore of great interests to identify resveratrol analogs with superior potency and better pharmacokinetic profiles.

Resveratrol trimethyl ether (*trans*-3,5,4'-trimethoxystilbene, RTE, also abbreviated as BTM-0512, MR-3 and TMS, Fig. 1 II) is a naturally

Abbreviations used: $AUC_{0\rightarrow 12h}$, Area under the plasma concentration-time curve from 0 to 12 h; Cl, Clearance; C_{max}, Maximal plasma concentration; C_{max}/Dose, Dose normalized C_{max}; CMC, Carboxymethylcellulose; F, Oral bioavailability; HP-β-CD, 2-hydroxypropyl-β-cyclodextrin; HPLC, High performance liquid chromatography; *MRT*, Mean residence time; P-gp, P-glycoprotein; RM-β-CD, Randomly methylated-β-cyclodextrin; RTE, Resveratrol trimethyl ether; t_{max}, Time to maximal concentration; $t_{1/2\,\lambda Z}$, Terminal elimination half-life; V_c, Apparent volume of distribution of the central compartment.

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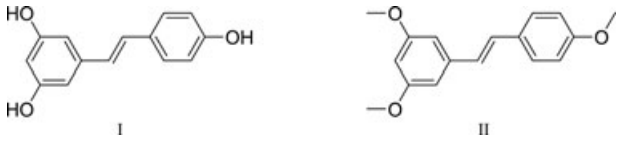

Figure 1. Chemical Structures of Resveratrol (I) and Resveratrol Trimethyl Ether (II)

occurring resveratrol derivative.[7,8] Its pharmacological activities have been reported recently. RTE exhibited anti-proliferative and/or apoptosis-inductive activities in various cancer cells with a potency usually higher than resveratrol.[9–17] Its *in vivo* anti-neoplastic effects were also confirmed in mouse xenograft models.[16,18] Moreover, RTE displayed anti-invasive and anti-angiogenic activities,[10,19–23] which may suppress tumor metastasis. Besides anti-cancer, RTE also possessed anti-allergic,[24] anti-diabetic,[25] anti-inflammatory,[25–28] gastro-protective,[29] and hepato-protective activities.[30] Clearly, RTE has emerged as a promising drug candidate for further pharmaceutical development.

Based on the chemical structure of RTE, improved metabolic stability can be predicted as all of its hydroxyl groups are protected by methoxylation. Furthermore, a fairly long terminal elimination and limited total plasma clearance have been observed in our preliminary study after single intravenous and oral administration.[31] In comparison with resveratrol, RTE is more liphophilic. The increased lipophilicity may cause two-edged effects on its pharmacology: on one hand, the lipophilicity increases the biomembrane permeability and subsequently, the pharmacological potency of RTE may be higher than resveratrol; on the other hand, the lipophilicity decreases the aqueous solubility of RTE and poses an issue on its oral bioavailability. Therefore, the impact of aqueous solubility on the oral absorption of RTE should be elucidated. Moreover, as RTE showed therapeutic potentials in several diseases required repeated drug treatment, its pharmacokinetic profiles after repeated administrations are crucial.

In this study, the pharmacokinetic profiles of RTE were investigated in Sprague-Dawley rats with the emphasis to identify the impact of aqueous solubility, dose escalation, food, and repeat dosing on its oral bioavailability. Our study provides important information to evaluate the suitability of RTE as a therapeutic agent for further drug development.

MATERIALS AND METHODS

Special Precaution

All experiments involving the manipulations of RTE and *trans*-stilbene were carried out in a dimly lit environment to prevent the photo-isomerization of stilbenes.[31]

Reagents

RTE (purity ≥ 97%) was obtained from Tokyo Chemical Industry (Tokyo, Japan). *trans*-Stilbene (internal standard, purity: 96%) and sodium salt of carboxymethylcellulose (CMC) were purchased from Sigma-Aldrich (St. Louis, MO). 2-hydroxypropyl-β-cyclodextrin (HP-β-CD, degree of substitution: about 0.6) and randomly methylated-β-cyclodextrin (RM-β-CD, degree of substitution: about 1.8) were generous gifts from Roquette (Lestrem, France) and Wacker (Burghausen, Germany), respectively. HPLC grade acetonitrile and methanol was obtained from Merck (Singapore) and Fisher Scientific (Fair Lawn, NJ), respectively. Purified water (18.2 MΩ·cm at 25°C) was generated from a Millipore Direct-Q® ultra-pure water system (Billerica, MA) and used throughout the study.

High Performance Liquid Chromatography (HPLC) Assay

The analysis was carried out on a Shimadzu (Kyoto, Japan) 2010A liquid chromatography with a RP-HPLC column (Agilent ZORBAX Eclipse Plus C18: 250 × 4.6 mm i.d., 5 μm), which was protected by a guard column (Agilent ZORBAX Eclipse Plus C18: 12.5 × 4.6 mm i.d., 5 μm). UV absorbance at 320 was used to quantify RTE. For sample cleanup, 150 μl *trans*-stilbene acetonitrile solution (concentration = 200 ng/mL) was added to 50 μl plasma. After vigorous vortex, the sample was centrifuged at 10,000 g at 4°C for 10 min. Finally, the supernatant was placed into a glass insert, which has been installed on an auto-sampler vial. The calibration curve, obtained by spiking RTE into pooled rat plasma, was linear (R^2 >0.998) within the range of 10–2000 ng/mL. The intra-day and inter-day variation were all less than 10% while the analytical recovery (%) and absolute recovery (%) of RTE in rat plasma ranged from 98–106%. In actual pharmacokinetic study, plasma samples with high RTE concentration (>2000 ng/mL) were properly diluted with blank plasma to within our calibration range (10–2000 ng/mL) before HPLC analysis. This HPLC assay was an improvement of our recently reported method.[31]

Preparation of Dosing Formulations

Cyclodextrins were used to form water-soluble formulations of RTE. As HP-β-CD is a parenterally safe excipient,[32] it was used to deliver RTE intravenously;[31] similarly, RM-β-CD usually offers stronger solubility-enhancive effect,[32] and it was therefore used in the oral formulation. Cyclodextrin based RTE solutions were prepared according to the following steps: (a) excess amount of RTE (HP-β-CD: 5 mg/mL; RM-β-CD: 15 mg/mL) was suspended in 0.3 M HP-β-CD or RM-β-CD solution; (b) the resultant