Food Chemistry 133 (2012) 683–688

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

# Inductive modulation on P-glycoprotein and cytochrome 3A by resveratrol, a constituent of grapes

Shih-Ying Yang [a], Shan-Yuan Tsai [b], Yu-Chi Hou [b,c,*], Pei-Dawn Lee Chao [b,*]

[a] Graduate Institute of Pharmaceutical Chemistry, College of Pharmacy, China Medical University, Taichung, Taiwan, ROC
[b] School of Pharmacy, China Medical University, Taichung, Taiwan, ROC
[c] Department of Medical Research, China Medical University Hospital, Taichung, Taiwan, ROC

ARTICLE INFO

*Article history:*
Received 1 September 2011
Received in revised form 24 November 2011
Accepted 23 January 2012
Available online 31 January 2012

*Keywords:*
Cyclosporine
Resveratrol
P-glycoprotein
CYP3A4

ABSTRACT

Resveratrol has been reported to show various beneficial pharmacological effects. Nowadays, resveratrol dietary supplement (RDS) is available in the market. Cyclosporin (CsA), a probe drug of P-glycoprotein (P-gp) and cytochrome P450 3A4 (CYP3A4), is an important immunosuppressant. This study investigated the effect of coadministration of RDS on CsA pharmacokinetics.

Rats were orally administered CsA alone and coadministered with RDS. The blood samples were assayed for CsA concentrations by a specific monoclonal fluorescence polarisation immunoassay method. Coadministration of 0.1 capsule/kg of RDS significantly decreased the $AUC_{0-t}$ and $C_{max}$ of CsA by 65% and 72%, and 0.2 capsule/kg of RDS reduced those by 78% and 84%, respectively. *In vitro* studies suggested that resveratrol enhanced the activities of P-gp and CYP3A4.

In conclusion, RDS decreased the absorption of CsA through induction modulation on P-gp and CYP3A. Transplant patients treated with CsA should be cautioned against taking RDS to reduce the risk of allograft rejection.



## 1. Introduction

Resveratrol (3,5,4′-trihydroxy-trans-stilbene, chemical structure shown in Fig. 1) is a polyphenolic compound found in grape skin, red wine (Jang et al., 1997; Siemann & Creasy, 1992) and other botanical sources including nuts (Cavallaro, Ainis, Bottari, & Fimiani, 2003) and Chinese herbs such as the root of *Polygonum cuspidatum* (Jang & Pezzuto, 1999). Resveratrol manifested antioxidation (Mikstacka, Rimando, & Ignatowicz, 2010; Soares, Andreazza, & Salvador, 2003), antibacterial (Maddox, Laur, & Tian, 2010), anti-inflammation (Kang et al., 2009), inhibition of vascular cell adhesion (Cottart, Nivet-Antoine, Laguillier-Morizot, & Beaudeux, 2010) and protection against ischemic injuries (Wang et al., 2002). Clinically, resveratrol has been found to be effective in cancer prevention (Jang & Pezzuto, 1999; Kundu & Surh, 2008). In addition, resveratrol may play positive roles in chemotherapy, including suppression of proliferation and invasion, arrest of cell cycle and induction of apoptosis (Sun et al., 2006). Because of the multiple pharmacological activities hitherto revealed, it is a rising star in the polyphenolic group and nowadays used as a dietary supplement by the public. However, owing to its abilities to modulate cytochrome P450 3A4 (CYP3A4) and P-glycoprotein (P-gp), the attentions on interactions with drugs have increased (Alvarez et al., 2010; Brand et al., 2006; Moon, Wang, & Morris, 2006).

Cyclosporine (CsA), a cyclic decapeptide, is a clinically important immunosuppressant, but with a narrow therapeutic window (Juliano Ling, 1976). Supratherapeutic blood level of CsA would cause adverse side effects such as nephrotoxicity, hepatotoxicity and neurotoxicity, whereas subtherapeutic blood level would spark off graft rejection for transplant patients (Burke et al., 1994). Therefore, how to maintain therapeutic blood concentration of CsA is a critical issue in clinical practise. The metabolism and transport of CsA were found to be associated with CYP3A4 and P-gp (Edwards et al., 1999; Goralski, Acott, Fraser, Worth, Sinal, 2006). Therefore, any modulator of P-gp or CYP3A4 may alter the pharmacokinetics and pharmacodynamics of CsA. This study investigated the effect of coadministration of resveratrol dietary supplement (RDS) on the pharmacokinetics of Neoral®, a microemulsion dosage form of CsA which was used as a probe drug of P-gp and CYP3A, in rats. Furthermore, the underlying mechanism involving P-gp and CYP3A4 in the interactions was explored by using *in vitro* models.

*Abbreviations:* CsA, cyclosporine; P-gp, P-glycoprotein; CYP3A4, cytochrome P450 3A4; RDS, resveratrol dietary supplement; HPLC, high-performance liquid chromatography; $C_{max}$, the peak blood concentration; $AUC_{0-t}$, the areas under the curves from time zero to last; MRT, mean residence time; $K_{10}$, elimination constant.

* Corresponding authors. Address: School of Pharmacy, China Medical University, No. 91 Hsueh-Shih Road, Taichung 40402, Taiwan, ROC. Tel./fax: +886 4 22031028 (Y.-C. Hou).

*E-mail address:* pdlchao@gmail.com (P.-D. Lee Chao).

0308-8146/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2012.01.069

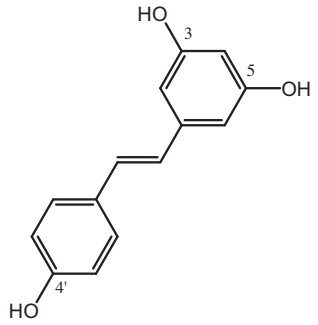


**Fig. 1.** Chemical structure of resveratrol.

## 2. Experiments

### 2.1. Chemicals and reagents

CsA (Neoral®, 100 mg/ml) was a kind gift from Novartis Co. Ltd (Taiwan). A commercial product of RDS was purchased from a dietary supplement store in Provo, Utah (Provo, UT, USA). The labelled content of resveratrol is 500 mg per capsule of RDS. Resveratrol (purity >98%) was purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). 3-(4′,5′-Dimethylthiazol-2′-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Alfa Aesar (Lancaster, UK). Rhodamine 123, sodium dodecyl sulphate (SDS), Triton X-100, verapamil, ethylparaben, sulfatase (type H-1 from *Helix pomatia*, containing 14,000 units/g of sulfatase and 498,800 units/g of β-glucuronidase) and L(+)-ascorbic acid were supplied by Sigma (St. Louis, MO, USA). Ethyl acetate was LC grade and purchased from Mallinckrodt Baker Inc. (Phillipsburg, NJ, USA). Methanol was LC grade and was purchased from Echo (Miaoli, Taiwan). Dulbecco's Modified Eagle Medium (DMEM), trypsin/EDTA, nonessential amino acid, Hank's Buffered Salt Solution (HBSS) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Invitrogen (Grand Island, NY, USA). Milli-Q plus water (Millipore, Bedford, MA, USA) was used for all preparations. TDx kit of CsA was purchased from Abbott Laboratories (Abbott Park, IL, USA).

### 2.2. Quantification of resveratrol in RDS and preparation of RDS suspension for dosing

The RDS capsules were undone the shells and extracted twice with 70% methanol. The supernatant (200 μl) was added with ethyl paraben solution (200 μl, 100 μg/ml in methanol as internal standard), and 20 μl was subject to HPLC analysis. An HPLC apparatus (Shimazdu LC-10AT, Japan) was equipped with an autoinjector (SIL-10A) and a photodiode array detector (SPD-M 10AVP) set at 280 nm. The column used is Apollo C18, 5 μm, 250 × 4.6 mm with a guard column (MetaGuard 4.6 mm Polaris 5 μm C18-A, MetaChem, Torrance). The mobile phase comprised 0.1% phosphoric acid and methanol (50:50), and the flow rate was 1.0 ml/min.

For calibrator preparation, the concentrations of resveratrol were in the range of 31.3–1000.0 μg/ml in methanol. The calibration curve was plotted after linear regression of the peak area ratios (resveratrol to the internal standard) with various concentrations of resveratrol.

Besides, five capsules were undone, the shells and the decanted powders were simmered with 50 ml of water and triturated in a mortar to afford RDS suspension (0.1 capsule/ml).

### 2.3. Animals and drug administration

Male Sprague–Dawley rats were provided by the National Laboratory Animal Center (Taipei, Taiwan) and reared in the Animal Center of China Medical University (Taichung, Taiwan). The animal study followed "The Guidebook for the Care and Use of Laboratory Animals (2002)" published by the Chinese Society of Animal Science, Taiwan. The protocol was approved by the Animal Management Committee, China Medical University. A total of 16 rats weighing 370–460 g ($n$ = 5–6 in each group) were fasted for 12 h before drug administration. Neoral® was diluted with water to afford 5.0 mg/ml of CsA. A dose of 2.5 mg/kg of CsA was given orally via gastric gavages with and without 0.1 and 0.2 capsule/kg of RDS (containing 46 and 92 mg/kg of resveratrol), which was given 30 min prior to CsA.

### 2.4. Blood collection and determination of blood CsA concentration

Blood samples of rats were withdrawn via cardiopuncture at 20, 40, 60, 180, 300 and 540 min after dosing of CsA. The blood samples were collected into small plastic vials containing EDTA, stored at 4 °C and assayed within one week.

CsA concentration in blood was measured by a specific monoclonal fluorescence polarisation immunoassay (Abbott, Abbott Park, III, USA). Validation of calibration curve was conducted by testing three controls before the sample assay. Otherwise, a new calibration curve would be constructed if necessary. The calibration range was 0.0–1500.0 ng/ml and the LLOQ was 25.0 ng/ml.

### 2.5. Cell line and culture conditions

LS-180, human colon adenocarcinoma cell line, was purchased from the Food Industry Research and Development Institute (Hsinchu, Taiwan). Cells were cultured in DMEM medium supplemented with 10 % foetal bovine serum (Biological Industries Ltd., Kibbutz Beit Haemek, Israel), 0.1 mM nonessential amino acid, 100 units/mL of penicillin, 100 μg/ml of streptomycin and 292 μg/ml of glutamine. Cells were grown at 37 °C in a humidified incubator containing 5% $CO_2$. The medium was replaced every two days and cells were subcultured when 80 to 90% confluency was reached.

### 2.6. Cell viability assay

The effects of resveratrol, verapamil and DMSO on cell viability were evaluated by MTT assay (Mosmann, 1983). Cells were seeded into a 96-well plate. After overnight incubation, the tested agents were added and incubated for 24 h. MTT (5 mg/ml) was added into each well and incubated for 4 h. In this period, MTT became formazan crystal by live cells. SDS solution (20%) was added to dissolve the purple crystal and the optical density was detected at 570 nm by a microplate reader (BioTex, Highland Park, Winooski, VT, USA).

### 2.7. Transport assay

The transport assay of rhodamine 123 followed a previous study with minor modification (Jia Wasan, 2008). LS-180 cells ($1 \times 10^5$) were cultured using a 96-well plate. The medium was removed and washed twice with ice-cold PBS after overnight incubation. Rhodamine 123 in HBSS (10 μM, 100 μl) was added to each well and incubated at 37 °C. After 1-h incubation, the supernatant was removed and washed twice with ice-cold PBS. Resveratrol, verapamil (as a positive control of P-gp inhibitor) and DMSO were added into correspondent wells and incubated at 37 °C. After 4-h incubation, the medium was removed and the cells were washed twice with ice-cold PBS. Then, 0.1 % Triton X-100 (100 μl) was added to lyse the cells, and the fluorescence was measured with excitation at 485 nm and emission at 528 nm. To quantify the content of protein in each well, 10 μl of cell lysate was added to 200 μl of diluted protein assay reagent (Bio-Rad, Hercules, CA, USA) and the optical density was measured at 570 nm. The relative intensity of