Available online at www.sciencedirect.com

ScienceDirect

Journal of Nutritional Biochemistry 24 (2013) 1823–1829

Journal of Nutritional Biochemistry

Absorption of resveratrol by vascular endothelial cells through passive diffusion and an SGLT1-mediated pathway☆,☆☆

Ming-liang Chen[1], Long Yi[1], Xin Jin, Qi Xie, Ting Zhang, Xi Zhou, Hui Chang, Yu-jie Fu, Jun-dong Zhu, Qian-yong Zhang, Man-tian Mi*

Research Center for Nutrition and Food Safety, Institute of Military Preventive Medicine, Third Military Medical University, Chongqing Key Laboratory of Nutrition and Food Safety, Research Center for Medical Nutrition, Chongqing 400038, People's Republic of China

Received 26 November 2012; received in revised form 1 April 2013; accepted 8 April 2013

Abstract

Resveratrol is a natural polyphenol that exerts potent effects to suppress atherosclerosis. However, its low concentration in plasma has placed this role in doubt. Thus, resveratrol effects might be dependent on its transport into vascular endothelium, a question not previously addressed in spite of its obvious and fundamental importance. Via high-performance liquid chromatography and liquid chromatography/mass spectrometry, we found that resveratrol was absorbed by human umbilical vein endothelial cells in a temperature-, concentration- and time-dependent manner, suggesting the involvement of passive diffusion and active transport. As determined by confocal laser scanning microscopy, resveratrol primarily distributed throughout the cytoplasm. Furthermore, resveratrol absorption was modulated by serum proteins and sodium-dependent glucose transporter 1 (SGLT1) yet inhibited by glucose (an SGLT1 substrate) and phlorizin (an SGLT1 selective inhibitor), as well as SGLT1 siRNA transfection. Additionally, Sprague–Dawley rats were intragastrically administrated with 100 mg/kg of resveratrol and the concentration of resveratrol in blood vessels declined more slowly up to 24 h compared to that in the blood. Our results suggested that resveratrol uptake by vascular endothelial cells involved both passive diffusion and an SGLT1-mediated process, at least partially. Moreover, the intracellular resveratrol pool may be more important than the serum level in vivo. These provide new insights into the cardiovascular benefits of resveratrol.

Keywords: Resveratrol; Vascular endothelial cells; Atherosclerosis; Sodium-dependent glucose transporter; Transmembrane transport

1. Introduction

Resveratrol (3,4′,5-trihydroxystilbene, RSV) is a natural polyphenol present in many fruits, vegetables and beverages, including red wine. Since the first report of the "French paradox," its potential protective role in preventing cardiovascular disease has been widely recognized [1]. Resveratrol administration ameliorated atherosclerotic plaque formation in hypercholesterolemic rabbits, which was ascribed to the inhibition of low-density lipoprotein oxidation, platelet aggregation and smooth muscle proliferation. As well, endothelial dysfunction, a first step in atherosclerosis (AS) progression, could be fundamentally inhibited by resveratrol through antioxidant and anti-inflammatory pathways [2–6].

The protective effects of resveratrol against cardiovascular disease were largely confirmed, yet recent investigations demonstrated that the bioavailability of resveratrol was a major concern for the development of this class of compounds into therapeutic agents [7,8]. It has been confirmed that resveratrol is mainly absorbed in the small intestine with high efficiency but very low bioavailability in humans due to rapid metabolism in vivo. Serum levels of unmetabolized resveratrol peak in the sub- to low-micromolar range, and the peak concentration is reached within the first 30 min [9–11]. Thus, the physiological effects of dietary resveratrol appeared to be in striking contrast to its poor bioavailability, unless attributable to yet unidentified accumulation in potential tissues or cellular sites. Moreover, various molecular targets of resveratrol have been identified in endothelial cells. Hence, the presence of membrane transporters in mediating rapid and specific absorption

Abbreviations: AS, atherosclerosis; BSA, bovine serum albumin; CLSM, confocal laser scanning microscopy; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; HPLC, high-performance liquid chromatography; HUVEC, human umbilical vein endothelial cell; LC/MS, liquid chromatography/mass spectrometry; MRM, multiple reaction monitoring; PBS, phosphate-buffered saline; SGLT1, sodium-dependent glucose transporter 1; SIRT1, silent mating type information regulation 2 homolog 1.

☆ Conflict of interest: None declared.

☆☆ Funding: This work was supported by the National Natural Science Foundation of China (grant number 81172670), Key Scientific and Technological Project of Chongqing (grant number CSTC, 2011AB5040) and the Youth Innovation Foundation of the Third Military Medical University (2012XJQ06).

* Corresponding author. Tel./fax: +86 2368752643.
E-mail address: mi_mt2009@hotmail.com (M. Mi).
[1] These authors contributed equally to this work.

0955-2863/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jnutbio.2013.04.003

of resveratrol by the endothelium remains unknown in spite of its obvious, fundamental importance. The only compelling evidence for entry of resveratrol into cells came from intestinal and liver cells, which was mediated through both passive diffusion and the sodium-dependent glucose transporter 1 (SGLT1)-mediated pathway [12,13]. SGLT1 is a member of the sodium-dependent glucose transporter family and is widely expressed in human cell membranes [14,15]. Recently, we found that SGLT1 was notably expressed in the vascular endothelial cells. Thus, we hypothesized that resveratrol might be absorbed by vascular endothelial cells through an SGLT1-mediated process.

The aims of the present study were to investigate resveratrol uptake and cellular distribution in vascular endothelial cells and define possible transport mechanisms. Our data revealed that resveratrol could be absorbed by vascular endothelial cells through both passive diffusion and an SGLT1-mediated mechanism. Further, despite its low bioavailability, we found that resveratrol was permanently available in blood vessels in vivo, which is important for its notable bioactivities.

2. Materials and methods

2.1. Chemicals and reagents

Cell culture media HyQ M199/EBSS (M199) and fetal bovine serum (FBS) were purchased from HyClone Laboratories (Logan, UT, USA). *Trans*-resveratrol, phlorizin (PLZ), glucose, dimethyl sulfoxide (DMSO), phosphate-buffered saline (PBS) and fetal bovine serum albumin (BSA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The SGLT1 antibody, fluorescein isothiocyanate (FITC)-conjugated secondary antibody, SGLT1 siRNA and control siRNA were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). 2-[^{3}H]Deoxy-D-glucose (6.0 Ci/mmol) was purchased from PerkinElmer Life and Analytical Sciences, Inc. (Boston, MA, USA).

2.2. Cell culture and treatment

Human umbilical vein endothelial cells (HUVECs) were isolated from umbilical cord veins as reported previously [16]. Cells were cultured on gelatin-coated plastic dishes with M199 medium supplemented with 20% FBS at 37°C in a humidified atmosphere containing 5% CO_2. All experiments were performed on the cells from 3 to 10 passage. During the logarithmic phase of growth, cells were treated with various concentrations (1 μM, 10 μM, 20 μM and 50 μM) of resveratrol for 2 h at 37°C or at 4°C. While cells were also treated with resveratrol (10 μM) for indicated time, control cells were cultured with 0.1% DMSO.

2.3. High-performance liquid chromatography (HPLC) and liquid chromatography/mass spectrometry (LC/MS) analysis

2.3.1. Sample preparation

At the end of incubation, cells were scraped from the wells, washed three times with cold PBS and then prepared according to procedures reported elsewhere [17,18] with some modifications. Briefly, cells were lysed by repeated shock freezing in liquid nitrogen and thawing, then combined with ethyl acetate and centrifuged at 14,000*g* for 20 min. The supernatant was collected and dried under nitrogen gas at room temperature. Dried extracts were suspended in 800 μl of methanol, and 20-μl aliquots were collected for the HPLC and LC/MS analysis.

2.3.2. HPLC analysis

The chromatographic analysis was carried out using an HPLC-UV-diode array detector (Waters^R 2487) and a C18 column (5 μm, 4.6 mm × 250 mm, ODS-3 100 Å Phenomenex Prodigy; Phenomenex, Torrance, CA, USA). The mobile phase (methanol, solvent A):water (solvent B) ratio was 48:52, and the flow rate was 1 ml/min. Resveratrol was identified by comparison of spectral features and retention time of the compound with a pure standard. Quantification, at a wavelength of 322 nm, was calculated using a calibration curve of pure standard compounds run under the same conditions.

2.3.3. LC/MS analysis

To confirm the identification and quantification of resveratrol internalization in endothelial cells, LC/MS was carried out as described previously with some modifications [19]. Briefly, LC/MS analysis was carried out using an Agilent 6410 Series Triple Quadrupole mass spectrometer (Agilent Technologies, Wilmington, DE, USA) equipped with an ESI source with the capillary voltage set at −4000 V and heated to 350°C.

Acquisition was carried out in the negative mode in multiple reaction monitoring (MRM) using deprotonated resveratrol (m/z 227) as a precursor ion. Therefore, the MRM transitions m/z 227→143.2 and 227→184.6 were selected to analyze resveratrol. The Agilent (Agilent Technologies) 1260 HPLC system was equipped with a G1322A vacuum degasser, a G1312B binary pump, a G1316B column oven and a G1367D autosampler. Chromatographic separation was performed on an Extend-C18 column (150 mm × 2.1 mm, i.d. 3.5 μm) (Agilent Technologies) protected by a flex capillary C18-A column (10 mm × 1.2 mm) (Agilent Technologies). The mobile phase containing methanol with 0.1% formic acid (phase A):water with 0.1% formic acid (phase B) ratio was 49:51 with a flow rate of 100 ml/min. The column was flushed with 100% phase B for 2 min to remove strongly retained compounds before re-equilibration with 10% phase B for 2 min.

2.4. Fluorescence microscopy

Cells were seeded into culture dishes at an optimal density of 1.6×10^4 cells per dish. After 24 h, the cells were exposed to resveratrol (10 μM) for 2 h at 37°C and then submitted to fluorescence spectral analysis via confocal laser scanning microscopy (CLSM). The excitation wavelength was set at 405 nm, and image sequences were acquired using a Leica TCS SPL equipped with a UV/visible laser (Spectra Physics 2018; Spectra Physics, Mountain View, CA, USA) and analyzed for emitted spectra from 410 to 500 nm.

2.5. Effect of serum proteins on resveratrol uptake

To investigate the interference of serum proteins on resveratrol uptake, HUVECs were incubated with 10 μM resveratrol in M199 supplemented with 20% FBS (as a control group) or in a serum-free media supplemented with different concentrations (0.5, 3.5 and 6 g/L) of BSA and incubated at 4°C for 2 h, which corresponds to a passive mechanism. The intracellular resveratrol content was measured by HPLC assay.

2.6. SGLT1 expression in HUVECs

About 1.6×10^4 cells were seeded on sterile culture dishes, paraformaldehyde-fixed and treated with 0.2% (v/v, in PBS) Triton X-100 for 10 min, and incubated for 1 h with blocking solution (5% BSA) at 37°C. Cells were cultured with anti-SGLT1 as a primary antibody (1:500 dilution) for 2 h at room temperature, and an FITC-conjugated secondary antibody (goat anti-rabbit IgG, 1:1000) was used to visualize immunocomplexes via fluorescence microscopy.

2.7. Determination of potential roles of SGLT1

2.7.1. Interference of sodium, glucose and PLZ on resveratrol uptake

To investigate the role of SGLT1 on transmembrane resveratrol transport in endothelial cells, the effects of sodium, glucose and PLZ on resveratrol uptake were determined. Briefly, HUVECs were seeded in 6-well plates and exposed to resveratrol (10 μM) in the presence or absence of sodium-free M199, glucose (10, 30 and 100 mM) and PLZ (0.25 and 0.5 mM) for 2 h at 37°C, respectively. Moreover, cells were also incubated with glucose (10 mM) in the presence of resveratrol (50 and 100 μM). The control cells were treated with 0.1% DMSO. Finally, the uptake of resveratrol in each group was measured as described in Section 2.3. Moreover, glucose uptake was essentially determined as previously described [20]. Briefly, cells were washed three times with 1-ml ice-cold PBS. Subsequently, cells were lysed with 0.5 ml of 0.5 M NaOH solution containing 0.1% SDS, and the solution was rotated for 15 min. Cell-associated radioactivity was measured by a liquid scintillation counter (PerkinElmer Life and Analytical Sciences, Inc.).

2.7.2. RNA interference

SGLT1 was knocked down by transfecting HUVECs with 40 nM siRNA according to the manufacturer's protocol. Briefly, after 5–7 h of transfection, cells were switched to M199 and incubated for 24 h and incubated with resveratrol (50 and 100 μM) or glucose (10 and 30 mM) for 2 h at 37°C. Thereafter, SGLT1 expression was determined by Western blotting according to our previously reported procedure [21] and the uptake of resveratrol and glucose in each group was measured as described in Section 2.7.1.

2.8. Animal study

2.8.1. Animals

Twelve female adult Sprague–Dawley rats (150–180 g) were housed in cages (n= 3/cage) under a 12-h light:dark cycle at 22±3°C and a relative humidity of 40–70% and received standard rat chow and water ad libitum. No traces of *trans*-resveratrol were detected in the commercial diet, as determined using the method reported by Juan et al. [17]. The experiments were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health and were approved by the Institutional Animal Care and Use Committee of the Third Military Medical University (Chongqing, China) (approval SYXC-2007-0002). Pentobarbital sodium anesthesia was administered prior to the surgical procedures, and all efforts were made to minimize suffering. Rats were fasted overnight and then randomly divided into two groups: an experimental group was administered a hydroalcoholic solution of resveratrol (100 mg/kg) by intragastric gavage, and a control group was administered the same volume of hydroalcoholic solution without resveratrol.