Nutrition, Metabolism & Cardiovascular Diseases (2013) 23, 1086–1092

Available online at www.sciencedirect.com

SciVerse ScienceDirect

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

Nutrition, Metabolism & Cardiovascular Diseases

Anti-estrogenic activity of a human resveratrol metabolite

R. Ruotolo [a], L. Calani [b], E. Fietta [a], F. Brighenti [b], A. Crozier [c], C. Meda [d], A. Maggi [d], S. Ottonello [a,**], D. Del Rio [b,*]

[a] *Laboratory of Functional Genomics and Protein Engineering, Biochemistry and Molecular Biology Unit, Department of Life Sciences, University of Parma, 43124 Parma, Italy*
[b] *The φ^2 Laboratory of Phytochemicals in Physiology, Human Nutrition Unit, Department of Food Science, University of Parma, 43125 Parma, Italy*
[c] *School of Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK*
[d] *Center of Excellence on Neurodegenerative Diseases, University of Milan, 20133 Milan, Italy*

Received 6 July 2012; received in revised form 19 December 2012; accepted 3 January 2013
Available online 5 March 2013

KEYWORDS
Resveratrol;
Resveratrol metabolism;
Phytoestrogen;
Red wine

Abstract *Background and aims:* Resveratrol, the most investigated dietary compound in studies aimed at linking wine consumption to human health, is an extremely minor component of this beverage and it is generally studied *in vitro* as the unconjugated aglycone at concentrations largely exceeding those found in the human circulatory system after dietary intake. Moreover, following intestinal absorption, *trans*-resveratrol and its glucoside, which are naturally present in wine and other food sources, are converted to sulphate and glucuronide metabolites. An estrogenic activity has previously been documented for resveratrol, yet nothing is known about the activity of its blood-circulating metabolic derivatives.
Methods and results: Using a yeast two-hybrid detection system relying on the interaction between the ligand-binding domain of the human oestrogen receptors α and β and the human coactivator Tif2, we have systematically examined the oestrogen agonist and antagonist activities of the two main resveratrol forms present *in planta* (*trans*-resveratrol and *trans*-resveratrol-3-*O*-glucoside) and of the three main metabolites found in human plasma (*trans*-resveratrol-3-*O*-sulphate, *trans*-resveratrol-3-*O*-glucuronide and *trans*-resveratrol-4′-*O*-glucuronide). Only resveratrol-3-*O*-sulphate was found to display a fairly strong and oestrogen receptor α-preferential antagonistic activity, which was confirmed in a human breast adenocarcinoma cell line containing a luciferase reporter gene under the control of an oestrogen-responsive promoter.

* Corresponding author. Tel./fax: +39 (0)521 033832.
** Corresponding author. Laboratory of Functional Genomics and Protein Engineering, Biochemistry and Molecular Biology Unit, Department of Life Sciences, University of Parma, Viale G.P. Usberti 23/A, 43124 Parma, Italy. Tel.: +39 (0)521 905646; fax: +39 (0)521 905151.
E-mail addresses: simone.ottonello@unipr.it (S. Ottonello), daniele.delrio@unipr.it (D. Del Rio).

0939-4753/$ - see front matter
http://dx.doi.org/10.1016/j.numecd.2013.01.002

Conclusions: We show, for the first time, that resveratrol-3-*O*-sulphate, but neither of its metabolites, is endowed with anti-estrogenic activity and how human metabolism of phenolic substances plays a pivotal role in modulating their biological effect.

The role of plant polyphenols as compounds responsible for some of the protective effects of a fruit-and-vegetable-rich diet has become an important area of human nutrition research. Unlike vitamins, polyphenols are not essential for short-term well-being. However, growing evidence indicates that a moderate long-term intake can favourably affect the incidence of various diseases including cancer and chronic diseases such as cardiovascular disease, type II diabetes and different neurodegenerative pathologies, which are becoming increasingly frequent in Western populations [1,2].

Resveratrol (3,5,4′-trihydroxy-stilbene; RES) is a secondary plant metabolite with a C6—C2—C6 structure. Two geometric isomers (*cis*- and *trans*-RES) and the corresponding 3-*O*-glucoside are produced by various edible plants — especially red wine grapes, peanuts and other nuts, red cabbage, spinach and some berries — in response to different types of stresses and growth conditions [3,4,5]. RES has been implicated in a variety of cellular processes, many of which are centred on the inflammatory cascade and on cell proliferation [6], and there are numerous claims in the press and in the scientific literature pointing to RES as the bioactive molecule responsible for the potentially protective effects associated with red wine consumption [7]. However, some of the proposed effects and purported targets of RES are still highly debated (see, for example, Ref. [8]). Another major, often neglected problem is that RES is an extremely minor component of red wine, and following ingestion it is converted to glucuronide and sulphate metabolites which are present in the circulatory system at nanomolar concentrations [9]. Nevertheless, the by far most commonly tested form of RES is the aglycone, often at concentrations largely exceeding those attainable *in vivo*. By contrast, very little is known about the biological activity of the RES metabolites formed upon intestinal absorption, which represent the major circulating forms of RES; in particular, the glucuronic acid and the sulphate conjugates of *trans*-RES which are produced at the enterocyte and hepatocyte level through the action of uridine 5′-diphospho-(UDP)-glucuronosyltransferases and sulphotransferases, respectively [10]. Thus, *in vitro* studies exclusively relying on the use of RES, at doses well in excess of the typical blood concentrations of its metabolites, are of very limited nutritional relevance.

In keeping with its structural similarity to the synthetic oestrogen diethylstilbestrol, RES has been shown to interact with human oestrogen receptors (hERs), with a slight preference for hERα, and has thus been designated as a 'phytoestrogen' (Ref. [11] and references therein). The functional consequences of such interaction are not straightforward as they depend on both the dose and the relative concentrations of RES and 17-β-oestradiol (E2), the main physiological ligand of hERs, and on the predominant type of hER (and associated coactivators) expressed by a given tissue or cell line [12,13,14]. It is now generally agreed that RES acts as a mixed agonist/antagonist at low concentrations, while it behaves as a pure anti-oestrogen at higher concentrations and in the presence of E2 [11,14,15]. However, while it is clear that at least some of the biological effects of RES are likely to be mediated by the interaction with oestrogen receptors, the relative functional importance of the RES aglycone and of its circulating metabolites as well as the specific phytoestrogenic activities of the latter compounds are as yet largely unknown.

To begin to address this question, the aim of this study was to evaluate, for the first time, the functional interaction of unconjugated RES, of its additional forms present *in planta* and of its human metabolites with hERα and β, using a second-generation yeast assay relying on the ligand-dependent recruitment of a transcriptional coactivator by either form of the hER.

Methods

Chemicals, yeast strains and mammalian cells

RES-3-*O*-glucoside was purchased from Sigma—Aldrich (St. Louis, MO, USA). *Trans*-RES, RES-3-*O*-glucuronide, RES-4′-*O*-glucuronide and RES-3-*O*-sulphate (R3S) were from Bertin Pharma (Montigny le Bretonneux, FRANCE). All liquid chromatography (LC) solvents were from Carlo Erba Reagents (Milan, ITALY).

Saccharomyces cerevisiae strain Y190 co-transformed with hERα ligand-binding domain (LBD) and hTif2 coactivator receptor-interacting domain expression vectors (pGBT9-ERα-LBD and pGAD424-TIF2, respectively) was a kind gift of Dr. Tsutomu Nishihara (Graduate School of Pharmaceutical Science, Osaka University, Osaka, Japan) [16]. The LBD of hERβ (amino acids 249—510) was polymerase chain reaction (PCR) amplified from the pMCV-hERß plasmid (kindly provided by Dr. Jan-Åke Gustafsson, Department of Biosciences and Nutrition, Karolinska Institutet, Stockholm, Sweden). The resulting amplicon was sequence-verified, cloned into the empty pGBT9 plasmid (pGBT9-ERβ-LBD) and transformed by electroporation into a modified Y190 strain, only carrying the pGAD424-TIF2 plasmid. Transformants were selected on synthetic defined (SD) medium lacking leucine and tryptophan.

The B17 clone of the human breast adenocarcinoma cell line, MCF-7, stably transfected with a luciferase reporter gene under the control of an oestrogen-responsive promoter [17], was used to verify the activity of R3S in mammalian cells.

Estrogenic and anti-estrogenic activity assays in yeast

The yeast two-hybrid (Y2H) assay was carried out as described [16,18] with slight modifications. Briefly, yeast