Identification and biological properties of new resveratrol derivatives formed in red wine

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1. INTRODUCTION

Wine is an important source of polyphenols both in terms of quality and quantity [1]. Among these compounds, resveratrol and its derivatives occupy a special place. This compound has long been considered one of the main responsible for the French paradox [2]. Resveratrol and its derivatives are well-known wine constituents, resveratrol and piceid being the most abundant [3, 4]. In addition, these compounds exhibited wide range of biological activities [5, 6]. Their content in wines depends on many biotic and abiotic factors intervening from grape to wine [7, 8]. The trans/cis isomerization of resveratrol under light exposure is a well-known chemical reaction that occurs in wine [9]. The hydrolysis of piceid by microorganisms was also described [10]. Moreover, resveratrol can be oligomerized by oxidative coupling reactions in presence of metals or enzymes [11, 12]. Recently, oxidative coupling of resveratrol was used to form oligomers with strong antifungal potential [13]. Moreover, we demonstrated that oxidative coupling of resveratrol occurs in wine leading to the formation of new dimers [14]. This article summarizes the main results obtained concerning the formation and presence of these compounds in wine. In addition, the biological activities of these compounds were also evaluated on cell lines.

2. MATERIALS AND METHODS

2.1. Oxidative coupling of resveratrol in ethanol

Oxidative couplings of resveratrol and piceid were conducted base on the protocol described in our previous work [14]. Briefly, resveratrol or piceid were stirred in presence of silver acetate (AgOAc) in ethanol under controlled conditions. Synthetized compounds were purified by liquid chromatography and identified by combination of mass and NMR spectrometry.

2.2. Wine treatment and analysis

Red wine samples were analyzed before and after heat treatment at 30°C for 24 hours. For resveratrol derivative analysis, wine samples were summited to solid phase extraction protocol using SPE Hypersep C18 cartridges (Thermo Fisher Scientific) as previously described [14]. Compounds identification and quantification were achieved by liquid chromatography coupled to triple quadrupole-mass spectrometry (LC-QqQ-MS) on an Agilent equipment. Analyses were performed in multiple reaction monitoring approach (MRM) using standard solutions for calibration curves.

2.3. Biological assays

2.3.1. Murine macrophage cell line assays

Anti-inflammatory effects were evaluated in RAW 264.7 macrophage cell line provided by the American Type Culture Collection (ATCC) as described else here [14]. Briefly, preventive effects of resveratrol derivatives against production of nitric oxide (NO) and reactive oxygen species (ROS) after exposition to lipopolysaccharide (LPS) were monitored.

2.3.2. Human breast cancer cell line assays

The human breast cancer (BC) cell lines MCF-7, HCC-1954 (estrogen- and progesterone-receptors negative) and MDA-MB-231 (triple negative) from ATCC, were used in cytotoxic assays. The cell lines were suspended in the incubation medium. HCC-1954 and MCF-7 were maintained in Roswell Park Memorial Institute medium (RPMI 1640), and MDA-MB-231 in Dulbecco's Modified Eagle's Medium (DMEM). Both culture media were supplemented with 10% heat inactivated fetal bovine serum, 2 mM L-glutamine and antibiotics (0.1 mg/mL streptomycin and 100 U/mL penicillin). DMEM was also supplemented with 1mM pyruvate. Cells were grown in an incubator at 37ºC with 5% CO_2 atmosphere. After reaching approximately 80-90% of confluence, cells were detached in a solution of 0.1% trypsin and 0.04% EDTA and plated as required for further experiments. Cell lines were seeded into 96-well plates at 3×10^3 (HCC-1954) and 2×10^3 (MCF-7 and MDA-MB-231) cells/well 24 h before treatment. Increasing concentrations (0-40 μM) of resveratrol, piceid, δ-viniferin, and δ-viniferin diglucoside were added and cells were incubated for 72 h. Stilbenes were dissolved in dimethyl sulfoxide (DMSO) at a final concentration of 0.02%. The same amount of DMSO was added to control cells. After treatment, the cell viability was determined using the crystal violet assay [16]. The absorbance was recorded at 590 nm in a Synergy HT microplate reader. Values were normalized with respect to the zero time control (no addition).

3. RESULTS AND DISCUSSION

3.1. Oxidative coupling of resveratrol and piceid

Resveratrol was subjected to oxidative coupling in presence of silver acetate in ethanol. This reagent is classically employed to induce phenol oxidation reaction [15]. The reaction of resveratrol leads to the formation of several resveratrol dimers including δ-viniferin, quadangularin B and oxistilbenin F and G [14]. Among these compounds, δ-viniferin is the principal dimer of resveratrol formed (Fig. 1).
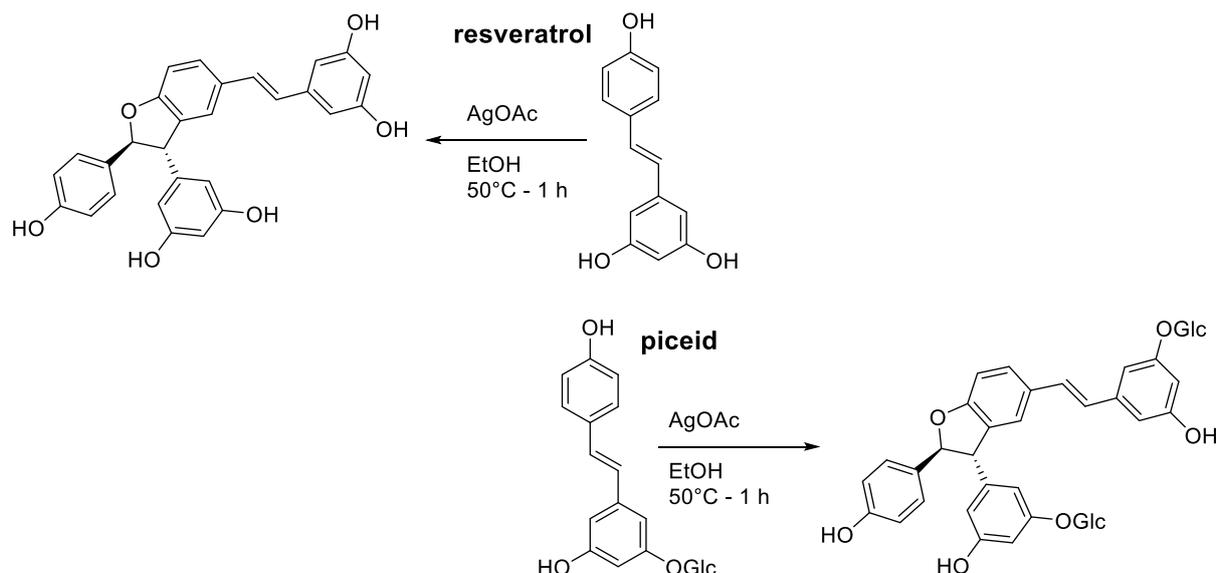
Figure 1. Dimerization of resveratrol and piceid in δ-viniferin and δ-viniferin diglucoside, respectively.

Similarly to resveratrol, piceid was subjected to oxidative coupling in presence of AgOAc in ethanol. The reaction leads to the formation of several products, δ-viniferin diglucoside being the most abundant (Fig. 1).

Compound identification was performed by purification on a preparative HPLC followed by mass spectrometry and NMR spectrometry. The δ-viniferin diglucoside was obtained as a brown amorphous powder. Complete structure elucidation was achieved by combination of HRMS and NMR experiments.

3.2. Formation of δ-viniferin in wine after heating

To verify whether oxidative coupling of resveratrol could occur in wine, a red wine was subjected to heating at 30°C for 24 h. Resveratrol and δ-viniferin contents were measured before and after heating (Fig. 2). Resveratrol derivatives were quantified on a LC-QqQ-MS spectrometer using MRM mode [14]. After heating, a significant decrease in resveratrol content is observed. This decrease is associated with a significant increase in δ-viniferin content. These results indicate that heating treatment for 24 h at 30°C induced oxidative coupling of resveratrol to form dimers. Thus, resveratrol dimerization could occur during wine aging.
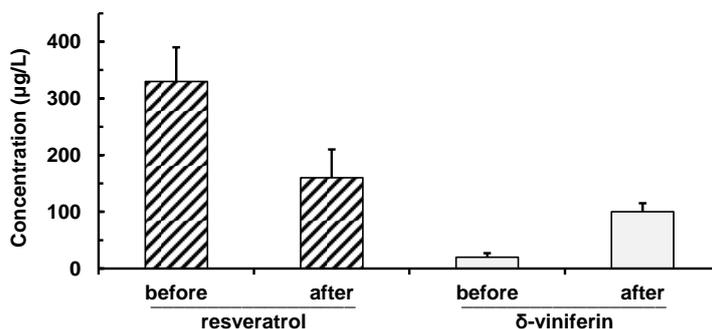
Figure 2. Resveratrol and δ-viniferin content before and after red wine heating (mean + SEM (n=3)).

3.3. Biological assays

The anti-inflammatory activities of dimeric stilbenes were evaluated using LPS-induce RAW 264.7 cells model. Resveratrol and δ-viniferin reduced NO and ROS production, δ-viniferin being the more active stilbene (Fig. 3). The δ-viniferin was slightly more active in inhibiting ROS production. The δ-viniferin diglucoside was not active. Thus, depending of the compounds, oxidative coupling could modulate the biological activities of red wine stilbenes.
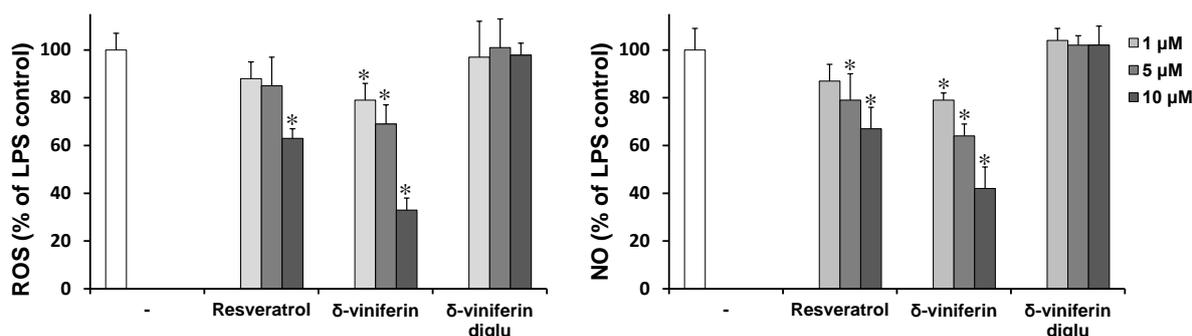
Figure 3. Effect of treatment with stilbene and LPS (0.1 µg/mL) on the ROS production and NO formation in RAW 264.7 cells. Data are expressed as percentage of the control (cells treated with LPS alone set to 100% production), corresponding to the mean ± SEM (n=4), *p<0.05.

In order to study the biological activity of different resveratrol derived stilbenes, human breast cancer cell lines were used as the biological model and the cell viability was determined by crystal violet assay. The compounds tested were resveratrol, piceid, δ-viniferin and δ-viniferin-diglucoside (Fig. 4). Results showed that resveratrol, the reference stilbene, was cytotoxic at the highest concentration of 40 µM in MCF-7 and MDA-MB-231, while it had no effect at lower concentrations or in HCC-1954. By contrast, the resveratrol-derived stilbenes increased cell growth to different degrees, depending on the cell type. In HCC-1954 the effect was significant and dose-dependent up for concentrations below 40 µM for the three compounds, at this concentration not being significant. In MCF-7, δ-viniferin diglucoside did not affect cell growth at any concentration, while piceid

increased proliferation dose-dependently. In MDA-MB-241, δ-viniferin had no effect on cell growth, while δ-viniferin diglucoside was proliferative at high doses. These preliminary results do not encourage the development of therapies based on these compounds against breast cancer.
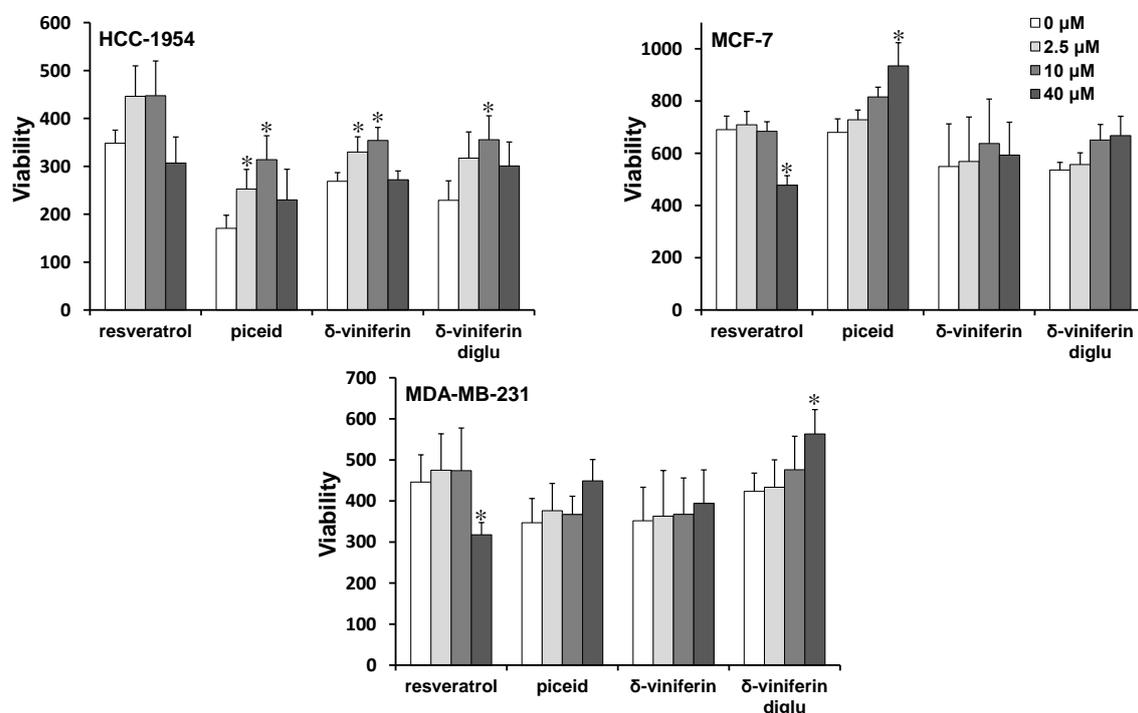
Figure 4. Comparison of stilbene effects on cell viability of human breast cancer cell lines: HCC-1954; MCF-7; and MDA-MB-231. Data are expressed as mean $\pm$ SEM (n = 3), *p<0.05 different from the corresponding control (no additions) at the same time.

Acknowledgments

References

1. R. Gutiérrez-Escobar, M.J. Aliaño-González and E. Cantos-Villar. Molecules 26 (3) (2021) 718.
2. B. Catalgol, S. Batirel, Y. Taga and N.K. Ozer. Front. Pharmacol. 3 (2012) 141.
3. R.F. Guerrero, J. Valls-Fonayet, T. Richard and E. Cantos-Villar. Food Control 108 (2020) 106821.
4. T. El Khawand, A. Courtois, J. Valls, T. Richard and S. Krisa. Phytochem. Rev. 17 (5) (2018) 1007.
5. N. Benbouguerra, R. Hornedo-Ortega, F. Garcia, T. El Khawand, C. Saucier and T. Richard. Trends Food Sci. Technol. 112 (2021) 362.
6. I. Aja, M. Begoña Ruiz-Larrea, A. Courtois, S. Krisa, T. Richard and J.I. Ruiz-Sanz. Antioxidants 9 (6) (2020) 469.
7. M. Poussier, M. Guilloux-Benatier, M. Torres, E. Heras, and M. Adrian. Am. J. Enol. Vitic. 54 (4) (2003) 261.
8. M.I. Fernández-Marín, B. Puertas, R.F. Guerrero, M.C. García-Parrilla and E. Cantos-Villar. J Food Sci. 79 (3) (2014) C310.
9. F. Mattivi, F. Reniero and S. Korhammer. J. Agric. Food Chem. 43 (7) (1995) 1820.
10. A. Roldán, V. Palacios, I. Caro and L. Pérez. J. Agric. Food Chem. 58 (7) (2010) 4268.
11. L. Wenling, T. Dong, P. Chen, X. Liu, M. Liu and X. Han. Tetrahedron 73 (21) (2017) 3056.
12. S.A. Snyder, A. Gollner and M.I. Chiriac. Nature 474 (2011) 461.
13. T. El Khawand, J. Gabaston, D. Taillis, M.L. Iglesias, Eric Pedrot, A. Palos Pinto, J. Valls Fonayet, J.M. Merillon, A. Decendit, S. Cluzet and T. Richard. OENO One 54 (1) (2020) 157.
14. T. El Khawand, J. Valls Fonayet, G. Da Costa, R. Hornedo-Ortega, M. Jourdes, C. Franc, G. de Revel, A. Decendit, S. Krisa and T. Richard. Food Res Int. 132 (2020) 109068.
15. V.S. Saraswati, I. Buniyamin, L. Kiew Ching, F. Feroz, I. Noorbatcha, L. Chuan Gee, K. Awang, I.Abd Wahab and J.F. Faizal Weber. Chem Eur J. 14 (36) (2008) 11376.
16. J. Trepiana, S. Meijide, R. Navarro, M.L. Hernandez, J.I. Ruiz-Sanz and M.B. Ruiz-Larrea. Redox Biol. 12 (2017) 103.