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Bioavailability and the mechanism of action of a grape extract rich in polyphenols in cholesterol homeostasis

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ABSTRACT

The bioaccessibility of a grape extract rich in polyphenols (GE) was determined in order to shed light on the mechanism of action in cholesterol homeostasis. GE's bioaccessibility and the antioxidant capacity of the bioaccessible fraction (BF) of GE were determined using a dynamic gastrointestinal digester (DGD). BF and GE were used to elucidate GE's mechanism of action, using two cell lines (HepG2 and Caco2) and different biomarkers. It was demonstrated that gastrointestinal digestion increases GE's bioaccessibility, with the antioxidant capacity of BF being statistically superior to that of GE ($p < 0.05$). With GE, a statistically significant increase in the expression of the low density lipoprotein receptor (LDLr) was noted, as well as for the intestinal cholesterol transporter (NPC1.L1) ($p < 0.05$). Besides, a statistically significant reduction in the expression of the cholesterol 7 alpha-hydroxylase enzyme (CYP7A1) ($p < 0.05$) was noted in BF.

1. Introduction

During the last few years, there has been a surge in interest when it comes to using and studying grape-derived polyphenols, given their antioxidant properties and their likely role in the prevention of several diseases linked to oxidative stress, such as cancer, cardiovascular disease and neurodegenerative disease (Manach, Scalbert, Morand, & Rémésy, 2004; Pastrana-Bonilla, Akoh, Sellappan, & Krewer, 2003; Sandhu, Gray, Lu, & Gu, 2011; Wang, Tong, Chen, & Gangemi, 2010). Thus, in order to establish conclusive evidence on the efficacy of

Chemical compounds: Ellagic acid (PubChem CID: 5281855); Gallic acid (PubChem CID: 370); Quercetin (PubChem CID: 5280343); Myricetin (PubChem CID: 5281672); (+)-Catechin (PubChem CID: 9064); (-)-Epicatechin (PubChem CID: 72276); Vanillic acid (PubChem CID: 8468); 5-(3′,4′-dihydroxyphenyl)-gamma-valerolactone (PubChem CID: 405103); Phenylacetic acid (PubChem CID: 999); Urolithin A (PubChem CID: 5488186).

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Abbreviations: GE, grape extract rich in polyphenols; ORAC, oxygen radical absorbance capacity; DGD, dynamic gastrointestinal digester; BF, grape extract bioaccessible fraction; LDLr, low density lipoprotein receptor; SREBP-2, transcription factor SREBP-2; CYP7A1, cholesterol 7 alpha-hydroxylase enzyme; NPC1.L1, intestinal cholesterol transporter

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polyphenols in the prevention of cardiovascular disease, their bioaccessibility (D'Archivio, Filesi, Varì, & Scazzocchio, 2010) must be verified and their mechanism of action charted.

The bioaccessibility of polyphenols is quite varied, and their effect on an organism can differ based on its composition and the characteristics of the matrix with which they are ingested. It is not always the case that the most plentiful matrix compounds are the ones that give way to greater concentrations of bioactive metabolites in tissue (Manach et al., 2004). Only the compounds that are released from the matrix are potentially bioavailable and can exert beneficial effects on the gastrointestinal tract (Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010; Fogliano, Corollaro, Vitaglione, & Napolitaro, 2011; Jin Hur et al., 2011; Bermúdez-Soto, Tomás-Barberán, & García-Conesa, 2007; Liang, Wu, Zhao, & Zhao, 2012; Cilla, Perales, Lagarda, & Barbera, 2011). *In vitro* digestion methods are useful for studying the release of these polyphenols and their stability under gastrointestinal conditions. This particular interest in the *in vitro* digestive trials resides in their faster pace, lower cost and in that they allow more control over experimental variables. Several studies have evaluated the effect of *in vitro* gastrointestinal digestion on the stability of pure phenolic compounds, but few have been performed while taking into account the effect of the matrix (Saura-Calixto & Díaz-Rubio, 2007). For the reasons detailed above, research on polyphenols' bioaccessibility of solid matrices utilising *in vitro* digestive methods are of particular interest. Even more so if these bioaccessible fractions are used to elucidate their mechanism of action on parameters associated with cardiovascular risk. In concrete terms, this study focused on cholesterol metabolism. To stipulate a precedence for the aforementioned focus, it should be mentioned that a prior study observed that the consumption of GE reduces LDL cholesterol levels (Yubero et al., 2013). Therefore, the aim of this study was to determine through which mechanism that effect was obtained. This is why it was deemed appropriate to address the different pathways for cholesterol metabolism, *de novo* synthesis, absorption and excretion.

In order to adapt the physiological processes to a laboratory scale, this GE called Eminol® was subjected to a sham treatment with the DGD as a prior step to its exposure to *in vitro* cell models. DGD allowed to determine Eminol's® bioaccessibility as well as the antioxidant capacity of BF. Subsequently, cell cytotoxicity tests were performed in order to identify the highest non-toxic concentration of BF suitable for use at cell level. This concentration of BF (and the equivalent concentration of GE) was used to analyse its behaviour upon key molecules for metabolising cholesterol. Two cell lines were used (HepG2 and Caco2) and the gene expression for the following biomarkers was analysed: SREBP-2, LDLr, CYP7A and 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoAR) for hepatic cells; and NPC1L1, for intestinal cells.

2. Materials and methods

2.1. *Grape extract*

The grape extract used in this trial was obtained from grape pomace. This extract, trademarked as Eminol® (ABROBIOTEC S.L., Ctra. San Bernardo S/N, 47359, Valbuena de Duero, Valladolid, Spain), was obtained by means of a unique and newly developed and patented system (P 2319032). The extract is 100% sourced from the *vitis vinifera* red grape variety – also known as Tempranillo – which is harvested from vineyards located in the region of Castile and Leon, in central Spain. These vineyards carry the *Ribera de Duero* Protected Designation of Origin. The polyphenol content for GE quantified by HPLC-MS/MS is shown in Table 1.

2.2. *High-pressure liquid chromatography (HPLC)*

The identification of polyphenolic compounds from GE was performed using HPLC-DAD-ESI-MS/MS and the quantification, using HPLC-DAD (Guerrero et al., 2009).

The method to perform this analysis was as follows: high-pressure liquid chromatography (HPLC) was carried out with a column Mediterranea C18, 5 µm 250 × 4.6 mm at 20 °C. Mobile phase A involved the use of distilled water/formic acid (95:5, v/v), whereas mobile phase B consisted in acetonitrile (100%). The flow rate amounted to 0.7 ml/min, and the elution was monitored at 280 nm.

2.3. *Dynamic in vitro gastrointestinal digestion*

A DGD (Viadel et al., 2012) was used to simulate *in vitro* digestion. A DGD is a multicompartment, computer-controlled system that simulates the biological environment in the human stomach and the small intestine. The secretion of digestive juices and the pH adjustment for each section are simulated according to physiological data.

The experiments were performed simulating the average physiological conditions in the upper gastrointestinal tract for healthy human adults, after consuming a meal (fed conditions). These conditions include the specific dynamics of peristaltic mixing, gastric emptying and small intestinal transit

Table 1 – Content of polyphenols in GE and amount of polyphenols recovered from GE in DGD.

	Gallic A.	Ellagic A.	Myricetin	Epicatechin	Catechin	Quercetin	Vanillic A.
GE	544 ± 6a	205 ± 58a	54 ± 15a	72 ± 17	248 ± 44a	143 ± 15a	247 ± 26a
Mastication	624 ± 10a	204 ± 10a	42 ± 30a	n.d.	9 ± 10b	154 ± 10a	105 ± 8b
Intestinal Digestion	541 ± 47a	159 ± 36a	35 ± 0a	n.d.	n.d.	116 ± 15b	122 ± 10b
BF	392 ± 57b	10 ± 30b	n.d.	n.d.	n.d.	n.d.	85 ± 11c

The results are expressed as mg of polyphenol/kg of GE. Media ± SD (n = 3) followed by the same letter (a, b, c, d) within a column are not significantly different ($p > 0.05$). n.d., not detected.