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Effects of red wine intake on human salivary antiradical capacity and total polyphenol content

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ABSTRACT

The protective effects of grape polyphenols have been reported on oral health, though unreasonable alcohol consumption represents a risk factor for developing oral cancer. The possible effects of red wine consumption on salivary antiradical activity were investigated in healthy volunteers for the first time, to the best of our knowledge. Time-course (from 0 min to 240 min) changes of salivary radical-scavenging capacity were measured by the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$) and 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) assays, in twelve healthy volunteers, after the intake of red wine (125 mL), a capsule of red wine extract (300 mg) or water (125 mL). Furthermore, time-course of salivary total polyphenol levels, detected by the Folin–Ciocalteu colorimetric method, was also determined. Both ABTS and DPPH tests showed that red wine consumption did not increase salivary antiradical activity in volunteers. Conversely, red wine extract administration caused a marked rise in salivary ABTS radical-scavenging capacity within 30 min, followed by a plateau up to 240 min. The same treatment also raised salivary DPPH radical-scavenging activity at any time point, though to a minor extent. The highest salivary polyphenol concentration was reached 30 min after wine drinking, followed by a steady decrease up to 240 min. Wine drinking was not associated to a reduced salivary antiradical capacity. However, wine extract greatly improved the salivary antioxidant status.

1. Introduction

In biological systems, free radicals, reactive oxygen and nitrogen species, may damage macromolecules, in a process known as oxidative stress. These harmful chemical intermediates are generated both unavoidably and deliberately, in cells and tissues, as result of mitochondrial oxidative metabolism and immune/inflammatory response, respectively. In addition, exposure to radiations, environmental pollutants (such as tropospheric ozone) and xenobiotics may contribute to exacerbate oxidative stress. Living organisms, both animals and plants, are able counteract the detrimental effects of these reactive species by their endogenous antioxidant defences, consisting of enzymes, high-molecular weight proteins and low-molecular weight scavengers distributed in diverse cell compartments, extracellular milieu and tissues. In humans, these barriers may be reinforced by exogenous dietary antioxidants, abundant in plant foods, whose healthy benefits have been widely reported. In any case, if not efficiently removed, reactive intermediates may irreversibly modify the structural and functional integrity of lipids, proteins and nucleic acids, process involved in the etiopathogenesis of the main chronic, degenerative disorders, including cardiovascular and neurodegenerative diseases and certain types of cancer (Lipinski, 2011; Sailaja Rao et al., 2011).

Total antioxidant capacity has been carefully measured in body fluids, plasma, saliva and urine, in both physiological and pathological conditions (Battino et al., 2002; Ziobro and Bartosz, 2003; Cornelli et al., 2010). In particular, in the oral cavity, saliva constitutes the first line of defence against oxidative stress, mechanistically involved in periodontal disease, precancerous lesions and oral cancer (Giebułtowicz et al., 2011). Saliva represents a complex and heterogeneous hypotonic fluid, a mixture of secretions from the major (submandibular, sublingual and parotid) and minor (accessory) salivary glands, together with gingival crevicular fluid (produced in the dentogingival sulcus), oral mucosa transudate, naso-pharyngeal secretions, gastrointestinal reflux, buccal epithelial cells,

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bacteria and their metabolites, blood cells and food debris. Healthy adults, normally, secrete 500–1500 mL of saliva per day, at a flow rate approximately ranging from 0.0 mL/min (during sleeping) to 6.0 mL/min (under stimulation), mainly composed of water (97–99%), electrolytes (Na^+, K^+, Ca^{2+}, Mg^{2+}, HCO_3^-, HPO_3^{2-}), proteins involved in lubrication (mucins), enamel remineralization (prolin rich proteins and statherins), starch digestion (α-amylase), innate immunity (immunoglobulins, defensins, lysozyme, lactoferrin, histatins, cystatins), cytokines (epidermal growth factor), albumins from plasma leakage, lipids (cholesterol, arachidonic acid), amines (polyamines and idoleamines) and hormones (cortisol, estrogens and androgens). Salivary flow and composition are under the control of the autonomic nervous system, depending on both parasympathetic and sympathetic stimulation (Caporossi et al., 2010). Similar to other biological systems, the salivary antioxidant defences consist of both non-enzymatic and enzymatic components, among which uric acid and peroxidases are the most efficient, respectively (Battino et al., 2002). Uric acid contributes to approximately 70% of the total salivary antioxidant capacity, reaching levels similar to that of plasma, with the role of ascorbate and gluthatione being less relevant. Salivary peroxidases are a complex system which includes lactoperoxidases, secreted by salivary glands, and myeloperoxidases, produced by polymorphonuclear neutrophils and macrophages. These enzymes catalyze peroxidation of thiocyanate and chloride, respectively, quenching H_2O_2 and generating antimicrobial products (Battino et al., 2002). Furthermore, as a diagnostic medium, saliva may provide useful data in diagnosing oral diseases (including malignancies), autoimmune disorders (such as Sjögren syndrome), systemic and infectious disorders (particularly human immunodeficiency virus, HIV, and *Helicobacter pylori*), and in monitoring exposure to xenobiotics and drugs (Caporossi et al., 2010).

Red wine is a relevant component of Mediterranean diet, a traditional nutritional style originated in areas where olive tree (*Olea europea* L.) and grapevine (*Vitis vinifera* L.) were cultivated, olive oil and wine produced and regularly consumed (Iriti and Vitalini, 2012). In the last decade, the beneficial effects of moderate alcohol consumption on health, especially in reducing the risk of cardiovascular diseases, have been reported (Costanzo et al., 2010). In particular, it has been suggested that the health-promoting properties ascribed to the regular, moderate red wine intake at main meals may be attributed to polyphenols, a complex group of natural compounds with many biological and pharmacological activities and abundant in food and medicinal plants (Iriti, 2011). Plant organisms are the only producers of these bioactive secondary metabolites, involved in their ecological relationships with the environment, mainly reproduction and defence. Biosynthesis of polyphenols arises from the essential aromatic amino acid phenylalanine, through the phenylpropanoid pathway, and they consist of three main groups: flavonoids (including anthocyanins), stilbenes (with resveratrol) and proanthocyanidins (or condensed tannins). Probably, antioxidant activity represents the paradigm of the healthy potential of red wine polyphenols (Iriti, 2011).

The protective effects of grape polyphenols have been also reported on oral health, though unreasonable alcohol consumption represents a risk factor for developing oral cancer, especially in association with tobacco smoking (Varoni et al., 2012). Ethanol may act as solvent for tobacco carcinogens, enhancing the permeability of oral mucosa, and, in addition, its metabolite acetaldehyde has been identified as a powerful procarcinogen (Seitz and Stickel, 2009). However, it seems that the carcinogenic effect of alcohol depends on drinking habits, including the amount and type of beverage. Heavy alcohol consumption has been associated with a major incidence of oral cancer, higher among spirit consumers than wine drinkers (Petti and Scully, 2005). Therefore, it is plausible that red wine, when correctly consumed and by virtue of its polyphenol content, may exert a protective effect on this malignancy, especially in the context of a Mediterranean dietary style (Rossi et al., 2007).

The aim of this study was to investigate the possible effects of red wine consumption on salivary antioxidant activity and polyphenol content. In order to better elucidate these aspects, we compared salivary antiradical capacity and total polyphenols in healthy volunteers, after administration of red wine in the form of beverage or extract. To the best of our knowledge, this study represents the first report on the effects of red wine intake on the salivary antioxidant status.

2. Materials and methods

2.1. Participants, experimental design and saliva collection

Twelve healthy adult Caucasian individuals (8 men and 4 women) with a mean age of 25.0 ± 1.0 years (ranging from 20 to 30 years of age) were enrolled among the students of the Dental Clinic of the Milan State University, where the study took place. They were of a middle/high-class socio-economic status and did not receive any remuneration for their participation at the study. The protocol complied with the Declaration of Helsinki and received the Institutional ethical approval; all volunteers were informed and authorization was obtained by signing a letter of consent before their enrolment into the study. The recruited participants had no clinical condition which could interfere with the results, and their dental and medical history was compiled. Their oral health status was carefully assessed by an accurate systematic examination of the oral cavity by the same dentist. None of the volunteers was taking any pharmacological treatment (antibiotics, anti-inflammatory or other drugs) or dietary supplement (vitamins, antioxidants or other phytochemicals) known to influence salivary secretion and total antioxidant status, as well as other variables of the study. All subjects were non-smokers, non-abstainers, non-professional or elite athletes, and their dietary habits included at least two and no more than four servings of fruits and vegetables per day. They were required to follow an appropriate diet the days before experiments and to fill in a questionnaire about their eating habits. Other exclusion criteria for volunteers included the presence of systemic and oral diseases; neurological disorders; diseases with possible effects on the immune system; pregnancy and lactation for women; consumption of more than 25–30 g/day of ethanol. Participants were instructed to refrain from consuming alcoholic drinks and polyphenol-rich foods and beverages two day before the saliva collection, and to avoid the use of any antibacterial mouthwash for 1 week before the experiment. Subjects fasted overnight and came to the laboratory at 08:30 am, where they sat and consumed a light polyphenol-free breakfast (40 g of non-whole biscuits with 125 mL of warm water). After brushing teeth for about 2 min without toothpaste, volunteers drunk 125 mL of red wine (Syrah IGT 2010, Sicily). Unstimulated whole saliva samples (3.0 mL) were collected by the spitting method (spitting saliva into a graded tube) from the volunteers at seven time points: just before (baseline) and 30, 60, 90, 120, 180 and 240 min, over 5 min periods with the subjects seated and instructed to allow saliva to pool in the bottom of the mouth and drain to the collection tubes. These latter were wrapped in aluminium foils and refrigerated at +4.0 ± 0.5 °C. Immediately after collection, saliva samples were centrifuged at 3000g for 10 min, at +4.0 °C, and the clear supernatant was stored in small aliquots at −80 °C until sample preparation for analyses. During the study, participants continued to execute their normal activity and did not consume any food or beverage After a wash-out period of 4 weeks, the study was repeated administering to the same individuals 125 mL of water (blank), and, after a further wash-out period of 4 weeks, they consumed a capsule of ethylcellulose with 300 mg (the maximum dose recommended by the manufacturer) of a commercial red wine extract (minimum 95% total polyphenols) ingested with 125 mL of warm water. After each treatment, a questionnaire was administered to record the participants' compliance.

2.2. Determination of salivary antiradical capacity

All the sample analyses were carried out in dim light conditions to avoid the photooxidation of bioactive metabolites. The 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) radical-scavenging activity was determined according to Atsumi and colleagues (1999). Absorbance was measured at 515 nm and the percentage of inhibition was calculated as $[(ABS_{control\ 517\ nm} - ABS_{sample\ 517\ nm}/ABS_{control\ 517\ nm}) \times 100]$.

The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$) radical cation-scavenging capacity was determined according to Re and co-workers (1999). The synthetic vitamin E analogue 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was used as positive control. Absorbance was measured at 734 nm and the results were expressed as Trolox equivalent antioxidant capacity (TEAC, mmol eq Trolox/mL saliva).