REVIEW-THEMED ISSUE

Dietary anthocyanins and health: data from FLORA and ATHENA EU projects

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Adherence to a 'Mediterranean diet' has been shown to be beneficial to human health. Fruit and vegetables represent some of the main components of the Mediterranean diet and their role has been increasingly considered in the process of preventing or reducing the risk of chronic degenerative diseases, such as cerebrovascular or coronary heart disorders, cancer and neurodegenerative diseases. To investigate the beneficial effect of these dietary compounds, two EU-funded projects were conducted during the last 10 years. Their results from experimental models suggest that dietary anthocyanin enrichment is beneficial against a number of ischemic and degenerative conditions. On the other hand, human studies demonstrated that anthocyanin supplementation can counteract the inflammatory response to stress conditions, such as a fatty meal. Moreover, an intervention trial in patients with breast cancer undergoing radiotherapy is presently testing the possible beneficial effect of the administration of a product enriched in anthocyanins on the inflammatory response to radiation and on its consequent skin toxicity, as well as on systemic low-grade inflammation reaction.

Introduction

Adherence to a 'Mediterranean diet' has been shown to be beneficial to human health. A population-based, prospective investigation involving more than 22 000 adults in Greece showed that greater adherence to the traditional Mediterranean diet is associated with a significant reduction in total mortality [1]. Sofi *et al.* [2] systematically reviewed all the prospective cohort studies that had analysed the relation between adherence to a Mediterranean diet, mortality and incidence of chronic diseases in a primary prevention setting and concluded that each two-point increase in a 9-point score reflecting the adherence to a Mediterranean diet is associated with a significant improvement in health status, as seen by a significant reduction in overall mortality (9%), mortality from cardiovascular diseases (9%), incidence of or mortality from cancer (6%), and incidence of Parkinson's disease and Alzheimer's disease (13%). These results seem to be clinically relevant for public health, in particular for promoting a Mediterranean-like dietary pattern for primary prevention of major chronic diseases. Large epidemiological studies show that a Mediterranean diet significantly reduces the risk of cardiovascular disease (by about −25%), of the same order of magnitude as pharmacological interventions [1, 2].

Fruit and vegetables represent some of the main components of the Mediterranean diet. The role of plant-based foods has been increasingly considered to prevent or reduce the risk of chronic degenerative diseases: in particular, dietary flavonoids have been associated with a reduction in cerebrovascular disorders and coronary heart disease (CHD).

In the 1990s, Renaud and de Lorgeril discussed the so-called 'French paradox': given a correlation between dairy fat consumption in diet and coronary heart disease mortality, reported from ecologic studies, inhabitants of France were observed to be outliers, with lower than expected levels of CHD. When age-standardized death rate for CHD in different countries and

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consumption of dairy fats was corrected for wine consumption, data concerning the French population were again compatible with the correlation [3]. The 'French paradox', although derived from observational data, had the merit of stimulating a great deal of research (3260 citations – according to Google Scholar – from 1992 to January 2016), improving the knowledge on the beneficial effects of wine and some of its non-alcoholic components preventing cardiovascular disease and other chronic disorders.

An experimental contribution of our group to this issue was an original study in rats reproducing the French paradox and supporting its hypothesis [4]. An artificial plastic loop was inserted into the abdominal aorta of rats. This loop became occluded in about 80 hours. Compared to animals fed a standard diet, rats fed a cholesterol-rich diet experienced a several-fold increase in lipid plasma levels with a concomitant significant increase in thrombotic tendency, measured as shortening of the occlusion time of the vascular prosthesis, and as platelet adhesion. Alcohol-free red wine supplementation to rats almost completely reverted the prothrombotic effect of the cholesterol-rich diet without changing the blood lipid levels. This experiment supports the concept that, despite dyslipidemia, intake of wine or of its non-alcoholic components is able to protect individuals at risk from ischemic events [4].

Different mechanisms have been investigated to explain the beneficial effects of bioactive polyphenols, of which the Mediterranean diet is particularly rich. It is generally agreed that these effects are mediated, at least in part, by antiplatelet and antioxidant activities of polyphenols, both contributing to downregulate the inflammatory response to injury [5–8]. The possible role on vascular function of resveratrol and of other non-alcoholic components of wine has recently been reviewed [9].

Flavonoids and health: experimental studies

Among polyphenols, anthocyanins belong to the bioactive family of compounds called flavonoids and are responsible for the red, purple, and blue colours of wine and many fruits, vegetables, cereal grains and flowers. These compounds are found in high amounts in blackcurrants, raspberries, aubergines, blood orange juice and blueberries. Although there are no clear indications for specific recommendations on the amount of flavonoids to consume on a regular basis, there is growing agreement that these bioactive compounds have beneficial effects on chronic diseases.

The need to provide sound mechanistic bases for the reported beneficial effect of these dietary compounds, prompted an interdisciplinary group of European investigators to start a joint venture which resulted in a ten-year collaboration through two subsequent EU-funded projects: FLORA (FLavonoids and related phenolics for healthy living using ORally recommended Antioxidants) and ATHENA (AnTHocyanin and polyphenol bioactives for Health Enhancement through Nutritional Advancement), respectively in the 6th (STREP 007130; from 2005 to 2009) and in the 7th (FP7-KBBE-2009-3; from 2010 to 2015) Framework Programs (http://athena-flora.eu). Both projects focused their attention on the possible health beneficial effects of anthocyanins.

Several European groups, coordinated by the John Innes Centre, Norwich, UK, participated in the project, with expertise ranging from plant physiology and genetics to preparation of foods enriched in anthocyanins, from analytical measurements of anthocyanins in food and biological matrices to cell biology, from experimental animal models of disease to human intervention studies. The main foods enriched in anthocyanins developed and tested in the projects were: tomatoes, obtained by genetic engineering [10], oranges (either rich or not in anthocyanins, red and blond, respectively) selected from different varieties [11], and corn (either rich or not in anthocyanins, black and yellow, respectively) obtained by classical breeding [12].

The possible protective effects of anthocyanin enrichment was tested in different experimental models: tomatoes were shown to extend lifespan in cancer-prone mice [10]; in other experiments, the size of an experimental myocardial infarction was significantly reduced in rats fed anthocyanin-rich corn [13]; supplementation with orange juice (rich in anthocyanins) reduced body weight gain and fat accumulation in mice, through mechanisms involving resistance to high fat diet-induced obesity and transcriptional reprogramming [14], and counteracted liver steatogenesis in mice with diet-induced obesity [15].

Our group contributed to the project first by the development and validation of a highly sensitive analytical LC–MS method to detect the main anthocyanin metabolites in biological fluids [16]. Through this method the bioavailability of anthocyanin metabolites was monitored both in the above-mentioned experimental models and in humans.

In healthy volunteers, four-week ingestion of red orange juice resulted in measurable urine levels of the three main metabolites, namely delphinidin-3-glucoside, cyanidin-3-glucoside and cyanidin-3-(6-malonylglucoside), reaching a plateau level after the first week of ingestion [17]. An expected high inter-individual absorption of the flavonoids was observed by the measurement of the plasma concentrations *vs.* time curve of anthocyanins in healthy volunteers after single administration of two-fold concentrated 500 ml of red orange juice; measurable urinary excretion of the same metabolites was also reported, and the same considered as markers of anthocyanin bioavailability in subsequent human studies [17].

Flavonoids and health: human studies

In healthy human volunteers chronic consumption of orange juice, rich (or not) in anthocyanins, did not affect platelet activation nor cell inflammation markers of cardiovascular disease [17], but decreased the procoagulant activity of whole blood: the latter effect was similar for both orange juices, either rich or not in anthocyanins, suggesting a role for antioxidants different from that of anthocyanins [18].

A single portion of orange juice in human volunteers was also effective in preventing the post-prandial inflammatory response to a standardized fatty meal [19]: the latter was previously shown to induce an acute, significant and reversible inflammation status, characterized by increased platelet and leukocyte counts and increased myeloperoxidase degranulation of granulocytes, all representative of an oxidative stress condition [20].

On the basis of these experiments, one could argue that the post-prandial condition, following a frequent fatty meal consumption, is a phenomenon that may lead to chronic low-grade inflammation and to a complex series of events (including endotoxemia) that may initiate and develop atherothrombosis [21]. Thus, a life-long adherence to a Mediterranean dietary pattern might favourably modulate low grade inflammation and reduce the risk to initiate and develop atherothrombosis [8, 22–24].

Moving from healthy subjects to patients, an observational study performed at the Catholic University in Campobasso reported that, in breast cancer patients, moderate wine consumption may have a protective effect on skin toxicity induced by radiotherapy, attributing this effect to the possible antioxidant effect of polyphenols contained in wine [25].

This observation, together with the experience gathered in studies on the anti-inflammatory effects of anthocyanins, prompted us to design an intervention trial in patients with breast cancer undergoing radiotherapy. The aim was to test the possible beneficial effect of the administration of a product enriched in anthocyanins on the inflammatory response to radiation and consequent skin toxicity, as well as systemic inflammation reaction, as evaluated by plasma biomarkers.

The synopsis of the protocol of the study, a randomized, placebo-controlled, double blind, monocentric evaluation, including 300 patients, is reported in the Supporting Information Table S1 (ClinicalTrials.gov ID: NCT02195960). The trial is ongoing and will be completed in the coming months.

Conclusions

The principal achievement of the FLORA and ATHENA research projects has been to include anthocyanins among the health-promoting dietary polyphenols that are effective in offering cardioprotection, protection against cancer and limitation of weight gain from obesity-inducing, high-fat diets, in preclinical studies with animals.

As human studies are more complex than experimental ones, the observed beneficial effects cannot be immediately translated into improvement of risk biomarkers for chronic disease in healthy volunteers, unless stress systems, such as the fatty meal, are used.

It is hoped that clinical intervention trials in subjects with risk factors or in patients undergoing a chronic inflammatory injury will provide the validation in humans of the health beneficial effects of the dietary modulation we have discussed.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: 1. no support from any organization for the submitted work; 2. no financial relationships with any organization that might have an interest in the submitted work in the previous three years; 3. no other relationships or activities that could appear to have influenced the submitted work.

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References

1 Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 2003; 348: 2599–608.

2 Sofi F, Cesari F, Abbate R, Gensini GF, Casini A. Adherence to Mediterranean diet and health status: meta-analysis. BMJ 2008; 337: a1344.

3 Renaud S, de Lorgeril M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet 1992; 339: 1523–6.

4 De Curtis A, Murzilli S, Di Castelnuovo A, Rotilio D, Donati MB, de Gaetano G, *et al.* Alcohol-free red wine prevents arterial thrombosis in dietary-induced hypercholesterolemic rats: experimental support for the 'French paradox'. J Thromb Haemost 2005; 3: 346–50.

5 Crescente M, Jessen G, Momi S, Höltje HD, Gresele P, Cerletti C, *et al.* Interactions of gallic acid, resveratrol, quercetin and aspirin at the platelet cyclooxygenase-1 level. Functional and modelling studies. Thromb Haemost 2009; 102: 336–46.

6 Pastori D, Carnevale R, Bartimoccia S, Nocella C, Tanzilli G, Cangemi R, *et al.* Does Mediterranean diet reduce cardiovascular events and oxidative stress in atrial fibrillation? Antioxid Redox Signal 2015; 23: 682–7.

7 Pignatelli P, Pastori D, Farcomeni A, Nocella C, Bartimoccia S, Vicario T, *et al.* Mediterranean diet reduces thromboxane A2 production in atrial fibrillation patients. Clin Nutr 2015; 34: 899–903.

8 Bonaccio M, Pounis G, Cerletti C, Donati MB, Iacoviello L, de Gaetano G. Mediterranean diet, dietary polyphenols and low-grade inflammation: results from the Moli-sani study. Br J Clin Pharmacol 2016; doi:. doi:10.1111/bcp.12924 [Epub ahead of print].

9 Gresele P, Cerletti C, Guglielmini G, Pignatelli P, de Gaetano G, Violi F. Effects of resveratrol and other wine polyphenols on vascular function: an update. J Nutr Biochem 2011; 22: 201–11.

10 Butelli E, Titta L, Giorgio M, Mock HP, Matros A, Peterek S, *et al.* Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. Nat Biotechnol 2008; 26: 1301–8.

11 Lo Piero AR, Puglisi I, Rapisarda P, Petrone G. Anthocyanins accumulation and related gene expression in red orange fruit induced by low temperature storage. J Agric Food Chem 2005; 53: 9083–8.

12 Petroni K, Cominelli E, Consonni G, Gusmaroli G, Gavazzi G, Tonelli C. The developmental expression of the maize regulatory gene Hopi determines germination-dependent anthocyanin accumulation. Genetics 2000; 155: 323–36.

13 Toufektsian MC, de Lorgeril M, Nagy N, Salen P, Donati MB, Giordano L, *et al*. Chronic dietary intake of plant-derived anthocyanins protects the rat heart against ischemia–reperfusion injury. J Nutr 2008; 138: 747–52.

14 Titta L, Trinei M, Stendardo M, Berniakovich I, Petroni K, Tonelli C, *et al*. Blood orange juice inhibits fat accumulation in mice. Int J Obes (Lond) 2010; 34: 578–88.

15 Salamone F, Li Volti G, Titta L, Puzzo L, Barbagallo I, La Delia F, *et al*. Moro orange juice prevents fatty liver in mice. World J Gastroenterol 2012; 18: 3862–8.

16 Giordano L, Coletta W, Rapisarda P, Donati MB, Rotilio D. Development and validation of an LC–MS/MS analysis for simultaneous determination of delphinidin-3-glucoside, cyanidin-3-glucoside and cyanidin-3-(6-malonylglucoside) in human plasma and urine after blood orange juice administration. J Sep Sci 2007; 30: 3127–36.

17 Giordano L, Coletta W, Tamburrelli C, D'Imperio M, Crescente M, Silvestri C, *et al*. Four-week ingestion of blood orange juice results in measurable anthocyanin urinary levels but does not affect cellular markers related to cardiovascular risk: a randomized cross-over study in healthy volunteers. Eur J Nutr 2012; 51: 541–8.

18 Napoleone E, Cutrone A, Zurlo F, Di Castelnuovo A, D'Imperio M, Giordano L, *et al*. Both red and blond orange juice intake decreases the procoagulant activity of whole blood in healthy volunteers. Thromb Res 2013; 132: 288–92.

19 Cerletti C, Gianfagna F, Tamburrelli C, De Curtis A, D'Imperio M, Coletta W, *et al*. Orange juice intake during a fatty meal consumption reduces the postprandial low-grade inflammatory response in healthy subjects. Thromb Res 2015; 135: 255–9.

20 Tamburrelli C, Gianfagna F, D'Imperio M, De Curtis A, Rotilio D, Iacoviello L, *et al*. Postprandial cell inflammatory response to a standardised fatty meal in subjects at different degree of cardiovascular risk. Thromb Haemost 2012; 107: 530–7.

21 Minihane AM, Vinoy S, Russell WR, Baka A, Roche HM, Tuohy KM, *et al*. Low-grade inflammation, diet composition and health: current research evidence and its translation. Br J Nutr 2015; 114: 999–1012.

22 Bonaccio M, Iacoviello L, de Gaetano G, Moli-Sani Investigators. The Mediterranean diet: the reasons for a success. Thromb Res 2012; 129: 401–4.

23 Bonaccio M, Di Castelnuovo A, De Curtis A, Costanzo S, Persichillo M, Donati MB, *et al*. Adherence to the Mediterranean diet is associated with lower platelet and leukocyte counts: results from the Moli-sani study. Blood 2014; 123: 3037–44.

24 Bonaccio M, Cerletti C, Iacoviello L, de Gaetano G. Mediterranean diet and low-grade subclinical inflammation: the Moli-sani study. Endocr Metab Immune Disord Drug Targets 2015; 15: 18–24.

25 Morganti AG, Digesù C, Panunzi S, de Gaetano A, Macchia G, Deodato F, *et al*.7 Radioprotective effect of moderate wine consumption in patients with breast carcinoma. Int J Radiat Oncol Biol Phys 2009; 74: 1501–5.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

http://onlinelibrary.wiley.com/doi/10.1111/bcp.12943/suppinfo.

Table S1 Synopsis of the study protocol of the ATHENA trial on anthocyanin supplementation in breast cancer patients undergoing radiotherapy