Rev Esp Cardiol. 2015;68(3):216–225

Original article

Polyphenol-enriched Diet Prevents Coronary Endothelial Dysfunction by Activating the Akt/eNOS Pathway

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Article history:
Received 13 November 2013
Accepted 1 April 2014
Available online 23 October 2014

Keywords:
Endothelium
Polyphenol-rich diet
Pomegranate extract
Nitric oxide
Vasodilation
Oxidative stress

ABSTRACT

Introduction and objectives: The Mediterranean diet, rich in polyphenols, has shown to be cardioprotective. However the mechanisms involved remain unknown. We investigated whether supplementation with a pomegranate extract rich in polyphenols renders beneficial effects on coronary function in a clinically relevant experimental model and characterized the underlying mechanisms.
Methods: Pigs were fed a 10-day normocholesterolemic or hypercholesterolemic diet. Half of the animals were given a supplement of 625 mg/day of a pomegranate extract (Pomanox®; 200 mg punicalagins/day). Coronary responses to escalating doses of vasoactive drugs (acetylcholine, calcium ionophore, and sodium nitroprusside) and L-NG-monomethylarginine (endothelial nitric oxide-synthase inhibitor) were measured using flow Doppler. Akt/endothelial nitric oxide-synthase axis activation, monocyte chemoattractant protein-1 expression, oxidative deoxyribonucleic acid damage in the coronary artery, and lipoprotein resistance to oxidation were evaluated.
Results: In dyslipidemic animals, Pomanox® supplementation prevented diet-induced impairment of endothelial relaxation, reaching vasodilatory values comparable to normocholesterolemic animals upon stimulation with acetylcholine and/or calcium ionophore. These beneficial effects were associated with vascular Akt/endothelial nitric oxide-synthase activation and lower monocyte chemoattractant protein-1 expression. Pomanox® supplementation reduced systemic oxidative stress (higher high-density lipoprotein-antioxidant capacity and higher low-density lipoprotein resistance to oxidation) and coronary deoxyribonucleic acid damage. Normocholesterolemic animals elicited similar drug-related vasodilation regardless of Pomanox® supplementation. All animals displayed a similar vasodilatory response to sodium nitroprusside and L-NG-monomethylarginine blunted all vasorelaxation responses except for sodium nitroprusside.
Conclusions: Pomanox® supplementation hinders hyperlipemia-induced coronary endothelial dysfunction by activating the Akt/endothelial nitric oxide-synthase pathway and favorably counteracting vascular inflammation and oxidative damage.

El enriquecimiento de la dieta con polifenoles previene la disfunción endotelial coronaria mediante la activación de la vía de Akt/eNOS

Palabras clave:
Endotelio
Dieta rica en polifenoles
Extracto de granada
Óxido nítrico
Vasodilatación
Estrés oxidativo

RESUMEN

Introducción y objetivos: La dieta mediterránea rica en polifenoles se ha demostrado cardioprotectora, pero se desconocen los mecanismos implicados. Se ha investigado los efectos de un extracto de granada rico en polifenoles en la función coronaria de un modelo porcino.
Métodos: Los animales ingirieron durante 10 días una dieta normocolesterolémica o hipercolesterolémica. La mitad de los cerdos recibieron un suplemento de 625 mg/día de un extracto de granada (Pomanox®; 200 mg punicalaginas/día). Se analizó (flujo-Doppler) la vasodilatación tras la administración coronaria de acetilcolina, ionóforo de calcio, nitroprusiato de sodio y L-NG-monometilarginina (inhibidor de la enzima óxido nítrico sintasa endotelial) y la activación del eje Akt/óxido nítrico sintasa endotelial, la expresión de proteína quimiotáctica de monocitos–1 y el daño oxidativo coronario del ácido desoxirribonucleico y la oxidación de las lipoproteínas.
Resultados: Pomanox® redujo la disfunción endotelial inducida por la dieta hipercolesterolémica a valores de animales normocolesterolémicos tras la estimulación con acetilcolina y/o ionóforo de calcio. Este efecto se asoció con mayor actividad coronaria de Akt/óxido nítrico sintasa endotelial, menor expresión de proteína quimioatáctica de monocitos–1 y menor daño oxidativo. Las lipoproteínas de alta

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http://dx.doi.org/10.1016/j.rec.2014.04.021
1885-5857/

densidad mostraron mayor capacidad antioxidante y las lipoproteínas de baja densidad fueron más resistentes a la oxidación. Pomanox® no afectó a la vasorrelajación de los animales normocolesterolémicos. Todos los animales mostraron similar vasodilatación tras la administración de nitroprusiato de sodio y la L-NG-monometilarginina bloqueó la vasorrelajación de todos los agentes vasoactivos, a excepción del nitroprusiato de sodio.

Conclusiones: La toma de Pomanox® previene la disfunción endotelial coronaria inducida por la hiperlipemia, al preservar el eje Akt/óxido nítrico sintasa endotelial y contrarrestar la inflamación y el daño oxidativo vascular.

Abbreviations

CVD: cardiovascular disease
eNOS: endothelial nitric oxide synthase
HC: hypercholesterolemic
HDL: high-density lipoproteins
LDL: low-density lipoproteins
NC: normocholesterolemic

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of mortality worldwide and atherosclerosis stands as one of its major underlying causes.[1] The endothelium plays a fundamental role in atherosclerosis prevention by regulating the vascular tone, leukocyte adhesion, and thrombus formation. In fact, endothelial dysfunction, believed to be a consequence of repeated exposure to cardiovascular risk factors (particularly hypercholesterolemia), is considered the hallmark of early atherosclerosis and is present even prior to the appearance of vascular lesions.[2,3] Furthermore, endothelial dysfunction has been shown to be a predictor of adverse outcome in patients with coronary artery disease.[4] Hence, strategies aimed at preventing or reducing endothelial damage have become a focus of attention.

Several epidemiological studies have evidenced that adherence to a healthy dietary pattern characterized by relatively high intake of fruits and vegetables is associated with a reduction in the incidence of CVD.[5,6] In fact, the existing data indicates that the role of fruits and their associated nutrients in cardiovascular prevention could be stronger than that of vegetables. In contrast, neutral and negative results have been obtained in controlled clinical trials failing to demonstrate significant CVD prevention with vitamin and antioxidant supplementations, underscoring the importance of whole foods.[7] Experimental and mechanistic evidence suggests that fruits present an array of disease-preventive phytochemicals, such as polyphenols, which contribute to the apparent modulation of atherosclerotic risk factors and atherosclerosis development.[8–10] In this regard, within the last decade, pomegranate (*Punica granatum L.*) has gained widespread popularity as a polyphenol-rich food with health-promoting properties.[11] Most of the pomegranate health benefits have been attributed to the presence of ellagitannins (mainly the large polyphenol compounds punicalagins isomers α and β), which are unique to pomegranate.[12] Although ellagitannins are not absorbed, under physiological conditions they become hydrolyzed to ellagic acid, which in turn is gradually metabolized by the intestinal microbiota to produce different types of urolithins (metabolites). Urolithins are thought to be responsible for the benefits associated with pomegranate consumption.[13,14]

In the present study we sought to investigate the *in vivo* effects of a pomegranate extract rich in punicalagins (namely Pomanox® [POX]) on vascular protection and to elucidate its underlying mechanisms. Indeed, whether pomegranate exerts vascular beneficial effects has yet to be determined. We carried out our study in a porcine model of coronary vasoreactivity fed either a regular chow or a high fat/high cholesterol-diet. Research using relevant animal models with translational clinical impact is needed to better determine and further explore the biological mechanisms through which polyphenol-rich foods may exert their clinical effects.

METHODS

The study protocol was approved by the institutional ethics committee *(Consejo Superior de Investigaciones Científicas-Institut Català de Ciències Cardiovasculars)* and all procedures fulfilled the criteria established by the "Guide for the care and use of laboratory animals" (National Institute of Health publication number 85-23, revised in 1996).

Study Design

Crossbred commercial female swine (48 [3] kg) were fed during 10 days a standard pig chow (normocholesterolemic [NC] diet, N = 12) or a high fat/high cholesterol diet (Western-type hypercholesterolemic [HC] diet, N = 12) of 20% saturated fat, 2% cholesterol, 1% cholic acid). We have already reported that intake of this fat-rich diet for 10 days raises cholesterol to levels comparable to that found in dyslipidemic humans and induces endothelial dysfunction.[15] Half of the NC and HC animals were provided a supplement of 625 mg/day POX. Four experimental groups (6 animals per group) were studied: NC; NC + POX; HC; and HC + POX. All animals were carefully monitored (ie, continuous supervision) to ensure the daily consumption of POX throughout the study. Pomanox® is a pomegranate extract standardized by its punicalagins α + β content. Pigs were supplemented during 10 days with a POX extract (punicalagins content of 32.21%), which corresponds to 200 mg punicalagins/day. This dose was chosen based on previous studies in humans, which used doses ranging from 78 mg/day punicalagins (tested in coronary artery disease patients supplemented during 3 years and diabetic patients during 3 months)[16] to 380 mg/day (healthy patients during 4 weeks).[17] The POX was provided by Probelte Biotecnologia S.L. (Spain).

On day 10, at 1 h post-dietary ingestion, coronary endothelium-dependent and –independent vasodilation was evaluated *in vivo* by catheter-based infusion of vasoactive substances into the left anterior descending coronary artery as previously described.[15] As to the treatment schedule, Seeram et al[18] reported that maximal plasma concentrations of a punicalagin-related metabolite, ellagic acid, were reached at 1 h after consumption of pomegranate fruit juice. At the end of the experimental procedure, animals were euthanized with an overdose of potassium chloride.