Nutrition, Metabolism & Cardiovascular Diseases (2006) **16**, 35–43

ELSEVIER

Nutrition, Metabolism & Cardiovascular Diseases

www.elsevier.com/locate/nmcd

Genistein affects the expression of genes involved in blood pressure regulation and angiogenesis in primary human endothelial cells

R. Ambra [a], G. Rimbach [b], S. de Pascual Teresa [c], D. Fuchs [d], U. Wenzel [d], H. Daniel [d], F. Virgili [a,*]

[a] *National Institute for Food and Nutrition Research, via Ardeatina 546, I-00178 Rome, Italy*
[b] *Institute of Human Nutrition and Food Science, Christian Albrechts University, Kiel, Germany*
[c] *School Food Biosciences, University of Reading, Reading, UK*
[d] *Department of Food and Nutrition, Molecular Nutrition Unit, Technical University of Munich, Germany*

Received 6 October 2004; received in revised form 8 March 2005; accepted 8 March 2005

KEYWORDS
Genistein;
Endothelial cells;
Gene expression;
Blood pressure;
Angiogenesis

Abstract *Background:* Several lines of evidence suggest that the dietary isoflavone genistein (Gen) has beneficial effects with regard to cardiovascular disease and in particular on aspects related to blood pressure and angiogenesis. The biological action of Gen may be, at least in part, attributed to its ability to affect cell signalling and response. However, so far, most of the molecular mechanisms underlying the activity of Gen in the endothelium are unknown.
Methods and results: To examine the transcriptional response to 2.5 μM Gen on primary human endothelial cells (HUVEC), we applied cDNA array technology both under baseline condition and after treatment with the pro-atherogenic stimulus, copper-oxidized LDL. The alteration of the expression patterns of individual transcripts was substantiated using either RT-PCR or Northern blotting. Gen significantly affected the expression of genes encoding for proteins centrally involved in the vascular tone such as endothelin-converting enzyme-1, endothelin-2, estrogen related receptor α and atrial natriuretic peptide receptor A precursor. Furthermore, Gen countered the effect of oxLDL on mRNA levels encoding for vascular endothelial growth factor receptor 165, types 1 and 2.
Conclusions: Our data indicate that physiologically achievable levels of Gen change the expression of mRNA encoding for proteins involved in the control of blood

* Corresponding author. Tel.: +39 06 51 494 517; fax: +39 06 51 494 550.
E-mail address: virgili@inran.it (F. Virgili).

0939-4753/$ - see front matter
doi:10.1016/j.numecd.2005.03.003

pressure under baseline conditions and reduce the angiogenic response to oxLDL in the endothelium.

Introduction

There is increasing evidence that dietary soy has health-promoting effects, particularly in relation to chronic diseases, such as coronary heart disease and cancer [1], which account for the majority of morbidity and mortality in industrialised countries. Soy products contain a significant amount of isoflavones, with genistein (Gen) being the most abundant [2]. The Food and Drug Administration (FDA) in the United States has recently approved a health claim for soy, since laboratory investigations, clinical trials and epidemiological data indicate that a high consumption of soy is associated with a lower incidence of coronary artery disease [3]. It is well known that vascular constriction is associated with increased risk of arteriosclerosis and hypertension. Several animal and clinical studies suggest that isoflavones can improve vascular compliance [4]. Soy isoflavones were shown to promote arterial dilatation and inhibit constriction in a group of female rhesus monkeys fed an atherogenic diet containing soy isoflavones [3]. Furthermore, dietary isoflavones improved systemic arterial compliance as compared to placebo, in a group of peri- and post-menopausal women [5]. A recent study of perimenopausal women fed isoflavones derived from soy protein showed a reduction in diastolic blood pressure [6]. In other human studies, a decrease in both systolic and diastolic blood pressure has been observed, in both men and women, following a long period of consumption of soy milk, and with a significant correlation between blood pressure reduction and urinary excretion of Gen [7].

Soy isoflavones are also thought to play a role in the prevention of vascular angiogenesis [8], but the mechanisms by which they mediate these beneficial effects have not been fully established. Angiogenesis is, at least in part, regulated by redox-sensing transcription factors and the expression of many genes associated with angiogenic response is likely to be regulated by antioxidants such as Gen. Furthermore, potential anti-angiogenic activities could account, at least in part, for the tumour preventive effects of Gen observed in prostate, breast and bladder cancer cells [8].

The analysis of differential gene expression in the endothelium is critical to the understanding of the sequence of events leading to the formation of high blood pressure, atherosclerotic lesions, smooth muscle proliferation, and angiogenesis. However, little is known about the effect of Gen on gene expression in human endothelial cells. Methods for large-scale measurement of gene expression [9] as well as proteomics [10] are becoming important techniques in the field of free radical research in endothelial function. Utilization of gene arrays is an expedient tool for ''*question driven*'' experiments [11] and intended to provide a hypothesis background for further targeted studies addressing the involvement of molecular pathways affected by a dietary treatment.

In order to gain a more comprehensive understanding of the molecular mechanisms of Gen, we analysed the gene expression profile of selected families of genes known to play an important role in molecular models of atherosclerosis. Some of the findings obtained by cDNA arrays, and relevant to the hypothesis of our study, were confirmed by using either RT-PCR or Northern blotting as independent techniques. Moreover, we tested the effect of Gen on endothelial cell response to a well-established pro-atherogenic stimulus, copper-oxidized LDL.

Methods

Cell culture

Primary human endothelial cells (HUVEC) were obtained from the umbilical cord vein according to the method of Jaffe and coworkers [12]. The vein of umbilical cords (kindly provided by the nursery of Department of Gynecological Sciences of the University of Rome ''La Sapienza'') was thoroughly washed with PBS and then incubated with 10 ml collagenase (0.2%, dissolved in Hanks medium) from *Clostridium histiolyticum* (type XI, Sigma, Poole, UK) for 20 min at 37 °C. The endothelial cell layer was removed from the vein using 50 ml of Hanks buffer (Sigma), subsequently pelleted by centrifugation at 800 × g for 10 min, and seeded in culture dishes pre-treated with 1.5% gelatine (type B from bovine skin, Sigma). A pool obtained from at least 10 different umbilical cords was subsequently grown in Medium 199 (Gibco Life Technology, Rockville, MD, USA) supplemented with 20% FCS, 2 mM glutamine, 1 mM sodium pyruvate, 10 mM Hepes, 100 μg/ml heparin, streptomycin–penicillin and 50 μg/ml endothelial cell