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Urolithins at physiological concentrations affect the levels of pro-inflammatory cytokines and growth factor in cultured cardiac cells in hyperglucidic conditions

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ABSTRACT

Diabetic cardiomyopathy (DCM) develops independently of common cardiovascular co-morbidities and is initiated by the metabolic derangements accompanying diabetes mellitus, including hyperglycaemia, which may cause a mild inflammatory state able to negatively affect myocardial biochemistry, structure, and function. This work shows how different urolithins, ellagitannin-derived metabolites, were able to modulate the pro-inflammatory mediators and growth factors secreted by rat cardiac myocytes and fibroblasts exposed to high glucose concentrations. At 1 μM concentration, coherent with dietary exposure to ellagitannin-rich foods, urolithins B and B-glucuronide succeeded in preventing inflammatory responses in cardiomyocytes, while in fibroblasts urolithin D was the most effective in controlling the overexpression of fractalkine, among the tested inflammatory mediators. Urolithins underwent extensive biotransformations in both cell types, including (de)glucuronidation, methylation, and sulphation. This suggests that the inflammatory bulk produced by hyperglycaemia could be attenuated by the regular intake of ellagitannin-rich foodstuffs such as pomegranates, raspberries, blackberries, strawberries, and walnuts.

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1. Introduction

Diabetes mellitus is associated with progressive cardiovascular diseases, including hypertension, coronary artery disease, and heart failure. Although many of these complications are secondary to diabetes-induced atherosclerosis, a non-atherogenic, specific cardiomyopathy, known as diabetic cardiomyopathy (DCM), has been recognised for over 30 years (Boudina & Abel, 2010; Bugger & Abel, 2014; Poornima, Parikh, & Shannon, 2006). Hyperglycaemia and dyslipidaemia play a pivotal role in the pathogenesis of DCM via a series of maladaptive stimuli among which cell oxidative stress and moderate inflammation are considered as key factors (Bugger & Abel, 2014; Giacco & Brownlee, 2010).

A mild inflammatory state, driven in part by activated M1 macrophages, constitutes an early pathophysiological condition in response to hyperglycaemia that may negatively affect myocardial biochemistry, structure, and function, favouring the onset of the DCM phenotype (Bugger & Abel, 2014; Huynh, Bernardo, McMullen, & Ritchie, 2014; Rajesh et al., 2010). Several molecular mechanisms have been proposed to contribute to the development of diabetic cardiomyopathy and a growing body of research has focused on novel strategies able to prevent hyperglycaemic-induced detrimental changes in the diabetic cardiac tissue (Rajesh et al., 2010). The diabetic milieu affects all cellular compartments of myocardial tissue, such as endothelial cells, myocytes and fibroblasts, and an altered activity of this latter cell type may trigger cardiac injury, playing a critical role in the occurrence of DCM (Fowlkes et al., 2013; Shamhart et al., 2009, 2014).

Urolithins, the main ellagitannin-derived microbial metabolites, exert potent anti-inflammatory activities in a broad array of cell and animal models at *in vivo* physiologically, attainable concentrations (Giménez-Bastida, González-Sarrías, Larrosa et al., 2012; Giménez-Bastida, Larrosa, González-Sarrías et al., 2012; González-Sarrías, Larrosa, Tomás-Barberán, Dolara, & Espín, 2010; Larrosa et al., 2010; Piwowarski, Granica, & Kiss, 2014; Piwowarski, Granica, Zwierzyńska et al., 2014; Verzelloni et al., 2011). Ellagitannins form a complex subclass of hydrolysable tannins present mainly in berries (raspberries in particular), pomegranate, walnuts, and oak-aged red wines (Zanotti et al., 2014) that have shown to exert different bioactivities (Li et al., 2015; Zhu, Nakagawa, Kishikawa, Ohnuki, & Shimizu, 2015). They are catabolised by the colonic microbiota to form a series of urolithins possessing a 6*H*-dibenzo[*b*,*d*]pyran-6-one structure with different phenolic hydroxylation patterns (Fig. 1) (Espín et al., 2007; González-Barrio, Edwards, & Crozier, 2011; Pfundstein et al., 2014; Tulipani et al., 2012). After absorption from the large intestine, urolithins appear in circulation as glucuronide, sulphate and methylated metabolites at concentrations ranging between high nM and low μM (García-Muñoz, Hernández, Pérez, & Vaillant, 2014). They can be accumulated in certain tissues (Nuñez-Sanchez et al., 2014), which may contribute to their potential preventive effects. Among other features related to inflammation, urolithins are able to inhibit tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6) production in THP-1-derived macrophages (Piwowarski, Granica, Zwierzyńska et al., 2014), down-regulate the levels of chemokine (C-C motif) ligand 2 (CCL2), plasminogen activator inhibitor-1 (PAI-1), and interleukin-8 (IL-8) in endothelial cells (Giménez-Bastida, González-Sarrías, Larrosa et al., 2012; Giménez-Bastida, Larrosa, González-Sarrías et al., 2012), and decrease the expression of microsomal PGE (prostaglandin E) synthase-1 (mPGES-1), PAI-1, IL-8, and cyclo-oxygenase-2 (COX-2), as well as inhibiting nuclear factor-kappaB (NF-κB) translocation to the nucleus in colon fibroblasts (Giménez-Bastida, González-Sarrías, Larrosa et al., 2012; Giménez-Bastida, Larrosa, González-Sarrías et al., 2012; González-Sarrías et al., 2010). However, not all the urolithins exhibit such bioactivities, and differences in their biological effects have been related to their structure (Giménez-Bastida, González-Sarrías, Larrosa et al., 2012; Giménez-Bastida, Larrosa, González-Sarrías et al., 2012; González-Sarrías et al., 2010; Piwowarski, Granica, Zwierzyńska et al., 2014; Verzelloni et al., 2011).

The aim of this work was investigating whether urolithins A, B, C, and D (Uro A, Uro B, Uro C, and Uro D) as well as a urolithin B-3-*O*-glucuronide (Uro B-gluc) (Fig. 1) could affect the levels of pro-inflammatory cytokines and growth factors related to the onset and progression of diabetic cardiomyopathy in cultured cardiac cells (cardiomyocytes and fibroblasts) maintained in hyperglucidic conditions.

2. Materials and methods

2.1. *Neonatal cell isolation and culture*

The study was conducted in conformity with the American Physiological Society guiding principles in the care and use of vertebrate animals in research and training. The protocol was submitted to, and approved by, the Veterinary Animal Care and Use Committee of the University of Parma (Prot. N° 59/12) and adheres to the National Ethical Guidelines of the Italian Ministry of Health.

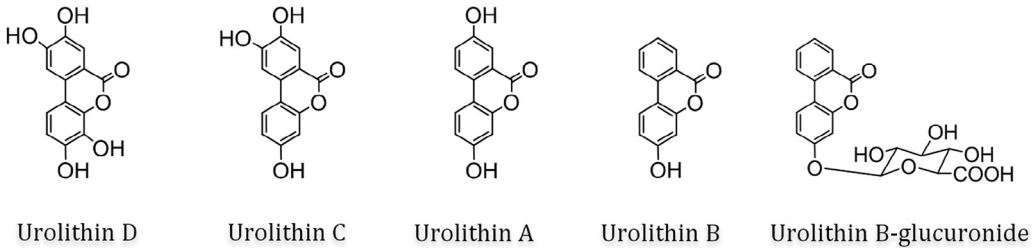

Fig. 1 – Chemical structures of urolithins.