Resveratrol for primary prevention of atherosclerosis: Clinical trial evidence for improved gene expression in vascular endothelium

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Resveratrol has been proclaimed as an anti-aging compound to prevent and treat chronic conditions, including cardiovascular disease, diabetes mellitus and neurodegenerative disorders [1]. Though resveratrol’s potential utility in preventive medicine has been demonstrated using animal models [2], few clinical trials have evaluated the effects of resveratrol on gene expression or clinically relevant biomarkers in healthy individuals. Trials evaluating cardiovascular risk generally measure plasma inflammatory biomarkers, including interleukins (IL) 1β, IL-6, Interferon Gamma (IFN-γ), Tumor Necrosis Factor alpha (TNF-α), but do not consider how components in plasma may interactively drive convergent endothelial cellular responses that contribute to the pathogenesis of atherosclerosis, including release of chemokines, such as IL-8 and activation of Vascular Cell Adhesion Molecule (VCAM) and Intercellular Adhesion Molecule (ICAM) [3–5]. This clinical trial evaluated the effects of one month resveratrol treatment on endothelial response and plasma biomarkers in healthy individuals using a novel unbiased assay to assess the overall inflammatory capacity of plasma on expression of genes associated with inflammation and atherosclerosis.

This double-blind, randomized, placebo-controlled clinical trial comprised 44 healthy subjects (ClinicalTrials.gov: NCT012444360). Informed consent was obtained from each subject and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution’s human research committee. Exclusion criteria were: age <18 years; history of significant general medical conditions; history of drug or supplement use which could alter metabolic or cardiovascular physiology; and use of grape-related supplements within one year. Subjects took either RESV (400 mg trans-resveratrol, 400 mg grapeskin extract, and 100 mg quercetin) or a cellulose placebo (PLA) for 30 days and were instructed to report side effects. Fasting blood was collected from each subject at baseline and the morning following the last day of treatment for biomarker assays.

Plasma concentrations of IL-1β, IL-6, IFN-γ, TNF-α, insulin, and leptin were analyzed using an electrochemiluminescence multiplex immunoassay (MesoScale; Lonza, NJ) were incubated with diluted plasma from participants. Gene expression of VCAM, ICAM, and IL-8 was quantified using our previously published methods [6]. Baseline versus post-treatment changes within each group were evaluated using paired t-tests when data met the assumptions of parametric statistics or the Wilcoxon signed-rank test when these assumptions were not met.

One subject did not complete baseline testing, another withdrew due to scheduling conflicts, and a third receiving PLA withdrew due to gastrointestinal side effects, resulting in 41 completers (RESV: n = 7 male, 13 female; PLA: n = 6 male, 15 female). Two RESV subjects and one PLA subject reported mild gastrointestinal side effects. Side effects were consistent with previously reported data [1].

As demonstrated in Fig. 1, exposing hCAECs to plasma drawn post-RESV resulted in significantly lower mRNA expression of VCAM (p = 0.017), ICAM (p = 0.037), and IL-8 (p = 0.022) than plasma drawn from the same subjects at baseline, whereas PLA had no significant effect (p = 0.65, 0.65, 0.753, respectively). There was a significant reduction in plasma IFN-γ in RESV (p = 0.033), but not in PLA (p = 0.96), and a significant reduction in fasting insulin concentration in RESV (p = 0.045 vs. p = 0.16 for PLA, one-tailed). These findings are in agreement with recent reports of resveratrol reducing inflammation and improving glucose tolerance in overweight subjects [7,8]. There was a trend toward increased IL-1β in PLA (p = 0.062), but not in RESV (p = 0.62). Fasting glucose, leptin, IL-1β, IL-6, and TNFα were within normal limits, and not significantly affected by either treatment. Lack of significant changes in plasma IL-1β, IL-6, and TNFα may be attributable to differences in subject population, dosage, and duration compared to other protocols [7,8].

Conventional plasma biomarkers of inflammation exhibited few changes, but endothelial cells were less activated by the complete plasma profile following RESV treatment. Various cytokines, proteins, and lipid intermediates converge mechanistically to influence the expression of ICAM, VCAM, and IL-8 [9], molecules that are directly related to atherosclerosis initiation [3,5]. RESV may have increased plasma concentration of resveratrol and its metabolites [1] within an individual, leading to a systemic milieu within plasma that significantly suppressed expression of these biomarkers. Endothelial cell responses may reflect changes in individual plasma components and/or complex interactions due to changes in multiple components which offer cardioprotective effects that may be otherwise undetectable using conventional biomarker assays. While ICAM, VCAM and IL-8 are involved in the promotion of atherosclerosis, the novel assay paradigm requires further characterization in terms of cardiovascular disease risk.

Our findings suggest that altered plasma composition leading to decreased expression of endothelial cell ICAM, VCAM and IL-8 may be an important mechanism contributing to resveratrol’s beneficial effects on cardiovascular function. Further, RESV may reduce
inflammatory biomarkers in individuals whose inflammatory biomarkers are already within normal limits, as demonstrated by decreases in plasma IFN-γ and insulin. Lastly, our novel assay demonstrates the potential value of evaluating the entire plasma milieu to quantify how various treatments alter endothelial response, and ultimately, atherosclerotic risk.

These data are the first to indicate that resveratrol may have protective effects against atherosclerosis in individuals who would not be considered high risk with the current screening criteria, suggesting that resveratrol supplementation could receive consideration as a primary preventive agent. We are in the process of designing and conducting a large-scale trial examining the effects of resveratrol supplementation to identify mechanisms of clinical benefits and how these effects can be maximized.

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

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References

Takotsubo and Takotsubo-like syndrome: A common neurogenic myocardial stunning pathway?

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Takotsubo diagnosis is currently established according to the widespread Mayo Clinic criteria [1], which excluded in its original version patients with concomitant intracranial haemorrhage or pheochromocytoma. Yet, a quite similar cardiomyopathy, defined as “neurocardiogenic stunning”, has been described in association with intracranial bleeding. Though originally considered a distinct entity, the updated version of the Mayo Clinic criteria integrate cerebral bleeding as a potential cause of Takotsubo Cardiomyopathy (TTC). On the other hand, pheochromocytoma remains an exclusion criterion for TTC diagnosis. However, growing data report transient left ventricular dysfunction syndromes (TLVDS) concurrent with catecholamine excess state, either exogenous, such as iatrogenic epinephrin-induced cardiogenic stunning [2], or endogenous, such as in pheochromocytoma. Similarity in clinical presentation and evolution between typical TTC and intracranial haemorrhage-related or pheochromocytoma-related stress cardiomyopathies led the Takotsubo Cardiomyopathy Study Group [3] to consider them as Takotsubo-like (TTL) syndromes. More broadly speaking, TTC and TTL may be considered a common subgroup of catecholamine-linked TLVDS. Blurred boundary between them illustrates TTC is a still being defined disease, which diagnostic criteria could be further discussed, both entities being merged into one category, i.e. catecholine-induced TLVDS.

We report here the case of a patient presenting with a pheochromocytoma-associated TTL, whose pathophysiologic basis were explored through nuclear medicine imaging.

A 52-year-old man presented to the emergency department with abrupt nausea, sweating and headache, concomitant with chest pain. No onset of emotionally stressful event was reported. Initial emergency room examination revealed blood pressure of 200/100 mm Hg, pulse 81 beats/min, and palpitations. Electrocardiogram showed alternating tachycardia-bradycardia with negative T waves in leads V1-V6 and D1-D2-aVL (Fig. 1A).

Initial laboratory work-up revealed elevated troponin T concentration 1 ng/mL (nl < 0.1), CPK 917 IU/L (nl < 190). Echocardiography displayed segmental antero-apical hypokinesis.

This overall presentation along with a past medical history of hypertension, type 2 diabetes and smoking, suggested the diagnosis of acute myocardial infarction (AMI). The patient thus underwent emergent cardiac catheterization, which showed no significant coronary artery abnormalities (Fig. 1B). Therefore, initial diagnosis was corrected to high blood pressure-linked AMI, with no coronary artery stenosis.

Because of the association of headache, sweating and nausea (Menard’s triad), pheochromocytoma was suspected and a 24-hour urine collection of catecholamines analyzed, detecting catecholamine hypersecretion, with epinephrine 1700 nmol (nl < 164), norepinephrine 3928 nmol (nl < 414) and dopamine 8682 nmol (nl 652–3260). An abdomen computed tomography (CT) scan displayed a 10 cm non enhanced right adrenal mass, with a hypodense necrotic central area, initially considered to be a hematoma.

Despite the CT-scan result, the clinical and biological presentation justified metaiodobenzylguanidine scintigraphy (MIBG-I131) scintigraphy, which was performed 2 weeks after the onset of symptoms. Whole-body planar acquisition, followed by single-photon emission computed tomography/CT (SPECT/CT), showed a focal intense tracer uptake with a central photopenic area, corresponding to the previously described right adrenal lesion. No extra-adrenal scintigraphic abnormality was observed.