reveratrol treatment, the
Fig. 2). A previous study
stimulated by resveratrol
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Identification and characterization of Tuscan extra-virgin olive oil extracts and their biological activity in rat VSMC.

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Abstract

In this work the effects of a defatted extract from extra-virgin olive oil (EVOO), void of vitamin E and rich in polyphenols, have been investigated in vascular smooth muscle cells (VSMC) freshly isolated from male and female rats. Different responses have been detected. We found that EVOO was able: i) to reduce lipid peroxidation in cells from male rats and to increase the content of total thiols in cells from female rats; ii) to decrease proliferation in both cell types; iii) to lower apoptosis in cells from male only; iv) to potently bolster autophagic cytoprotection, mainly in cells from female. These results suggest that EVOO might exert differential effects on the homeostasis of vascular cells from males and females rats.

Introduction

Extra virgin olive oil is one of the main components of the Mediterranean diet. Its beneficial health effects are attributed to its high content of monounsaturated fatty acids (particularly oleic acid) [1] and to content of polyphenol micronutrients called minor polar compounds (MPC). Extra virgin olive oil is obtained directly from pressing ripe olives and retains sizeable amounts MPC and tochopherols (Visioli and Galli, 2001), which can act as antioxidants (Mateos et al, 2003). Indeed, the qualitatively and quantitatively composition depends on many factors such as olive cultivar, and agronomic and technological aspects of production (). In this contest, it is important to recall that a) the mixture of phenols may exert different activity in comparison with the single phenols, because they may cooperate thereby modifying biological activity (Reaven and Witzum, 1996) b) interactions among phenols seems also depend on the relative amount of single polyphenols (Romani et al, 2004). Thus, individual extra virgin olive oil, which differ qualitatively and quantitatively and could have different biological activities. Considering that MPC exert antihypertensive effect [1,7-8] we evaluated a defatted extract from extra-virgin olive oil (EVOO), void of vitamin E and rich in MPC in VSMC studying apoptotic process, a relevant process in the vessel remodeling, and autophagy, a critical factor in the maintenance of cell homeostasis [17].

Materials and Methods

The extraction of MPC, as well as the identification, characterization and quantification of single polar compounds, were carried out as reported by Brunelleschi et al. [6].

Plasma low density lipoprotein (LDL) was isolated from plasma of healthy human donors and oxidized with 5μM $CuSO_4$ as reported by Giovannini et al. [13]. VSMC were isolated from the descending aorta of young female and male rats; cultured as described by Coinu et al., [14] and treated with 10 μM EVOO for 24h and with 100mg ox-LDL protein/l for 72h. Cell count was performed by using a Coulter Counter. Apoptosis were performed by flow cytometry. SOD and catalase were measured spectrophotometrically by using commercial kits. Western blot analysis was used to evaluate autophagy. Cytofluorimetric results were analyzed by non-parametric Kolmogorov-Smirnov test using Cell Quest Software. For cell growth curves statistical analysis was performed by using the parametric Tukey HSD test.

Results and Discussion

The MPC composition was identified and quantified in the extract of EVOO. Four classes of phenolic compounds were detected: simple phenols 10.38 mM (tyrosol and 5-hydroxytyrosol), secoiridoids 15.64 mM (oleuropein aglycone, deacetoxy-oleuropein aglycone, oleocanthal, and secoiridoid derivatives), lignan derivatives 2.64 mM (acetoxypinoresinol) and flavanols 0.17 mM (luteolin). The extract was free of vitamin E and squalene, and the total concentration of polyphenols was about 34 mM. We show for the first time that the Tuscan EVOO, at a concentration of MPC comparable to that obtained *in vivo* after consumption of a dietary amount of EVOO,[15,16], exerts differential effects on VSMC isolated from males rats.

Moreover, after 72h of treatment, EVOO decreased cell growth rate and protected from the apoptosis induced by ox-LDL, considered as a "physiological" pro-oxidant and pro-apoptotic stimulus. Considering the importance of autophagy in the maintenance of cell homeostasis [17] the expression of two autophagic specific biomarkers was taken into consideration: a) the soluble form of LC3 (LC3I) and its converted form (LC3II/ATG8) localized in autophagosomal membranes, and b) Beclin 1 (ATG5), a component of the phosphoinositide 3-kinase complex that seems to play an important role during the initial steps of autophagosome formation. Interestingly, the EVOO treatment modified the autophagic process. Specifically, it reduced LC3I and increased LC3II expression thus improving the autophagic behavior. In summary, EVOO improves autophagy response and reduces apoptotic process induced by oxLDL. This could represent an important adaptive response in modifying vessel remodelling that play a crucial role in hypertension

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