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Modulation of the superoxide anion production and MMP-9 expression in PMA stimulated THP-1 cells by olive oil minor components: Tyrosol and hydroxytyrosol

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ABSTRACT

Extra virgin olive oil has been associated with a reduced incidence of risk factors for coronary heart disease also owing to the presence of antioxidant biophenols. Reactive oxygen species (ROS) and matrix metalloproteinase-9 (MMP-9) have been implicated in numerous somatic illnesses, including cardiovascular disorders and cancer. The aim of this work was to study the capacity of virgin olive oil tyrosol (T) and hydroxytyrosol (HT) at impairing superoxide production and MMP-9 expressions in monocyte cells (THP-1) conveniently differentiated into adherent macrophages, taken as a model of human macrophages implicated in atheroma.

$O_2^{\bullet-}$ production was evaluated in THP-1 cells by using lucigenin as a specific chemiluminescent probe. Cells, after differentiation for 72 h, were preincubated in the presence of HT and T at increasing concentrations for 4, 15 and 24 h, and then, monocyte-like cells were stimulated by phorbol myristate acetate (PMA) and the $O_2^{\bullet-}$-dependent luminescence was immediately recorded at 37 °C by means of a Luminometer. Enzymatic activity of MMP-9 derived from a medium of cells preincubated, or not, with T or HT was tested by zymography.

As compared to the cells without treatment, cells preincubated with HT, showed a decrease of $O_2^{\bullet-}$ production (50%) at 1 μM for 15 h of preincubation time. Tyrosol fully prevented ROS overproduction at 15 h and, like HT displayed a high degree of protection but at higher concentrations and later time points (24 h). Gelatin zymograms revealed a reduction of the expression of MMP-9 in conditioned medium derived from T and HT-treated cells. These findings give further evidence in favour of olive oil consumption to counteract cardiovascular diseases.

1. Introduction

Reactive oxygen species (ROSs) are toxic products formed as a result of a decrease of molecular oxygen in the cell (Barbieri, Eligini, Brambilla, Tremoli, & Colli, 2003; Chinetti et al., 1998). Activated macrophages, especially in the atherosclerotic lesion, are a major source of reactive oxygen species (ROS). In monocytes/macrophages, ROS sources are the mitochondrial electron transport chain, cyclo-oxygenases, lipo-oxygenases, cytochrome P-450 and nicotinamide adenosine dinucleotide phosphate oxidase (Barbieri et al., 2003; Chinetti et al., 1998). The change of redox form of the cell induces the activation of a series of proteins and intracellular enzymes (epidermal growth factor receptor, c-Src, p38 mitogen-activated protein kinase, Ras, and Akt/protein kinase B) (Baas & Berk, 1995) as well as transcription factors redox-sensitive (NF-κB and AP-1) (Paravicini & Touyz, 2006) and the consequent induction of genes involved in the endothelial functions (adhesion molecules and prothrombotic factors) and in the change of the extracellular matrix that is involved in the formation and progression of the atherosclerotic disease. Excessive extracellular matrix breakdown by matrix metalloproteinases (MMPs) is implicated in many physiological and pathological conditions, particularly in inflammatory status such as atherosclerotic lesion development, cell infiltration and proliferation (Dell'Agli, Canavesi, Galli, & Bellosta, 2005). In particular, gelatinases, such as the 72-kDa gelatinase A (MMP-2) and the 92-kDa gelatinase B (MMP-9), are abundantly expressed in various malignant tumors, play an active role in angiogenesis, and may also influence the process of atherosclerotic lesion formation and destabilization (Dell'Agli et al., 2005). Previous studies showed that MMP-9 is present in atheroma and abdominal aortic aneurysm (Herron et al., 1991) and elevated levels are associated with stroke or cardiovascular death (Eldrup, Gronholdt, Sillesen, & Nordestgaard, 2006).

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The formation of ROS is balanced by a range of antioxidant defenses, but the excess of ROS attacks and damages virtually all biomolecules in the cells, leading to cell death and serious chronic diseases such as cancer, heart disease, and cerebrovascular disease (Lee et al., 2009; Scandalios, 2005). A possible way to prevent ROS-mediated cellular injury is to augment endogenous oxidative defenses through the dietary intake of antioxidants (Di Mascio, Murphy, & Sies, 1991; Krinsky, 1991). Dietary phenolic antioxidants, ubiquitous in vegetables, fruits and their juices possess antioxidant activity that may have beneficial effects on human health (Manach, Scalbert, Morand, Remesy, & Jimenez, 2004; Pearson, Tan, German, Davis, & Gershwin, 1999). In fact, it has been reported to be inversely related to mortality from coronary heart disease and to the incidence of myocardial infarction (Kris-Etherton & Keen, 2002; Williamson & Manach, 2005). Phenolic compounds have been shown to possess free radical-scavenging and metal-chelating activities in addition to the reported anticarcinogenic properties (Middleton, 1998). Experimental studies demonstrated that LDL oxidation is substantially reduced by consumption of several phenolic compounds (Fuhrman, Lavy, & Aviram, 1995; Hodgson et al., 2000). Furthermore, the antiatherogenic effect of polyphenols has also been ascribed to the observed capacity of these molecules to reduce platelet aggregation (Petroni et al., 1995).

Extra virgin olive oil, the typical added fat of the Mediterranean diet, has been associated with a reduced incidence of risk factors for coronary heart disease (Harwood & Yaqoob, 2002; Manna et al., 1999; Nakbi et al., 2010; Owen et al., 2000; Visioli, Poli, & Galli, 2002). This is partly due to the high amount of monounsaturated fatty acids (Svegliati et al., 1999), but it is likely to be also the result of the presence of phenolic compounds that have strong antioxidative power (Mateos, Dominguez, Espartero, & Cert, 2003). The major phenolic compounds in olive oil — oleuropein, hydroxytyrosol (HT) and tyrosol (T) — are strong antioxidants and radical scavengers (Tuck & Hayball, 2002) that can help revert the imbalance between increased ROS production and impaired antioxidant defense that affects endothelial function and therefore contributes to atherosclerotic disease progression (Perona Javier, Cabello-Moruno, & Ruiz-Gutierrez, 2006).

The aim of this paper was to investigate the possible protective effect of extra virgin olive oil HT and T against ROS-mediated oxidative injuries and MMP-9 expression in PMA stimulated THP-1 human monocyte after differentiation into an adherent macrophage taken as a model of human macrophages implicated in atheroma.

2. Materials and methods

2.1. Materials

Retinoic acid, IFNγ, phorbol-12 myristate-13 acetate (PMA), lucigenin (10, 10′-dimethyl-9,9′-bisacridinium dinitrate) were purchased from Sigma Chemical Co. (St Quentin Fallavier, France). Cell culture medium and fetal calf serum (FCS) were from Bio Media (France). Hydroxytyrosol (HT) was from Claire Dufour Avignon and tyrosol (T) was from Fluka, USA. All other current chemicals were obtained from Merck.

2.2. Cell line and culture

The human THP-1 cell line was grown in RPMI 1640 medium containing 0.2 mmol/l glutamine, 10 U/ml antibiotics and 10% FCS, 5% CO_2 at 37 °C. The THP-1 cell line was used after a 72 h differentiation time period, which led to major cell alterations (such as the adhesion capacity and the capacity at producing superoxide anion $O_2^{\bullet-}$). Differentiation was performed in the presence of 10^{-6} M retinoic acid, 10^{-7} M 1α, 25-dihydroxycholecalciferol (vitamin D_3), and 100 units/ml IFNγ (Shafiee, Carbonneau, Urban, Descomps, & Leger, 2003). This differentiating mixture was used instead of PMA in order to avoid the downregulating cell effect of prolonged PMA incubation time on PKC and PKC-dependent events (including superoxide production). Then, the culture medium was removed and replaced by RPMI 1640 without the differentiating agents and macrophage differentiated monocytes were incubated further at 37 °C with the substances to be tested.

2.3. Superoxide anion production and measurement

Once differentiated, cells were incubated in the absence or presence of the increasing concentrations of HT (1, 5, 10 and 50 μM) and T (0.05, 0.15, 0.5 and 2 mM) for 4, 15 and 24 h and then removed from the culture medium before PMA (0.1 μM) addition. The production of $O_2^{\bullet-}$ by macrophage was measured at 37 °C with lucigenin-amplified chemiluminescence (10^4 M lucigenin) by means of a microplate luminometer (EGG Berthold, France). The luminescence signal was counted over the 90-min period following PMA addition. In the control conditions, RPMI medium only contained the solvent vehicle used for polyphenols dilution (ethanol, not exceeding 0.2% in the medium).

2.4. MMP-9 expression

MMP-9 enzymatic activity in conditioned medium was determined by SDS-PAGE gelatin zymography. Zymography was performed using polyacrylamide gels containing 1 mg/ml gelatin. Concentrated conditioned medium ((5 μl, containing equal amounts of protein) was loaded on the gel and electrophoresed at 4 °C for 135 min at 120 V. After electrophoresis, gels were washed twice in 50 mM Tris–HCl, pH 7.5, 0.15 M NaCl, 2.5% Triton X-100 for 30 min in order to remove SDS. Proteolytic activity was revealed by incubation of the gel in the buffer at 37 °C for 24 h. After fixation and Coomassie blue staining, enzyme activity was identified as clear bands against the blue background. The gel was scanned and the digitised picture was further contrasted using Paintshop-Pro software. The density of each band was evaluated using the software Scion image and the data was expressed as histogram form.

2.5. Statistical analysis

Results were given as means ± S.D. The value for each experiment corresponded to the means of eight determinations for superoxide anion production. Non paired Student's t was used for statistical analysis. $P \leq 0.05$ was considered statistically significant.

3. Results

3.1. Effects of biophenols on superoxide anion production

Monocytic line THP-1 after differentiation into adherent macrophages, taken as a model of human macrophages implicated in atheroma, is able to produce $O_2^{\bullet-}$ after PMA stimulation. Figs. 1 and 2 showed the effect of HT and T on anion superoxide production by PMA-stimulated macrophages after preincubation for 4, 15 and 24 h at increasing concentrations. In fact, the preincubation of the cells with HT and T for 4 h didn't show an impairment of $O_2^{\bullet-}$ production in PMA stimulated cells (Figs. 1A and 2A). However, the preincubation during 15 h significantly lowered the quantities of superoxide produced in cells as well for HT and T. A significant decrease was observed at 1, 5 and 50 μM for HT (Fig. 1B) and at 0.15 mM for T (Fig. 2B).

After an additional 24 h incubation time with increasing biophenol concentrations, the PMA stimulated cells showed a significant decrease of $O_2^{\bullet-}$ production only at higher concentration of HT (50 μM) compared to cells without preincubation (Fig. 1C). As illustrated by Fig. 2C, T was able to dose-dependently inhibit the anion superoxide production by PMA stimulated macrophages.