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Lipid peroxidation in plasma of rats treated with Fe-NTA: protective effect of the phenolic fraction of extra virgin olive oil

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Titolo
Perossidazione lipidica nel plasma di ratti trattati con Fe-NTA: effetto protettivo della frazione fenolica dell'olio extravergine d'oliva

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Ferric-nitrilotriacetate, lipid peroxidation, phenolic compounds

Parole chiave
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Summary
It has been reported that the phenolic fraction (PF) of extra virgin olive oil contains compounds that are important inhibitors of lipid peroxidation *in vitro* and are believed to be effective through their free radical scavenging and metal chelating properties. An excellent model of *in vivo* free radical induced damage, associated with extensive lipid peroxidation, is the ferric-nitrilotriacetate (Fe-NTA) model. Intraperitoneal injection (i.p.) of Fe-NTA leads in rats to increasing oxidative stress, that starts from the plasma compartment, where Fe-NTA finds the ideal environment to react with oxidizable lipids, as unsaturated fatty acids (UFA) and cholesterol in lipoproteins particles. To investigate the action of the PF as inhibitor of the lipid peroxidation process in the plasma compartment, we treated Wistar rats with the PF (25 mg-50 mg/Kg bw) prior to the administration of a sub-lethal dose (15 mg Fe/Kg bw) of Fe-NTA. Fe-NTA treatment induced a significant decrease of UFA and cholesterol, together with an increase of fatty acids hydroperoxides (HP) and 7-ketocholesterol (7-keto). I.p. administration of PF significantly inhibited fatty acids and cholesterol oxidation, and reduced the levels of HP and 7-keto. Phenolic compounds may associate with lipoproteins particles, increasing their resistance against oxidation or may directly trap radicals generated in the plasma aqueous compartment.

Riassunto
La frazione fenolica dell'olio extravergine d'oliva (PF) comprende una serie di composti inibitori della perossidazione lipidica *in vitro*, che agiscono da scavenger di specie radicaliche o chelanti dei metalli. Un interessante modello sperimentale di danno radicalico *in vivo*, associato ad un'estesa perossidazione lipidica è il modello del nitrilotriacetato ferrico (Fe-NTA). La somministrazione intraperitoneale di Fe-NTA provoca nei ratti un forte stress ossidativo, che interessa inizialmente il compartimento plasmatico, dove il Fe-NTA trova l'ambiente di reazione ideale per reagire con i lipidi più suscettibili all'ossidazione, come gli acidi grassi insaturi (UFA) e il colesterolo delle particelle lipoproteiche. Per valutare l'azione protettiva della PF contro la perossidazione lipidica nel comparto plasmatico, abbiamo trattato ratti Wistar con la PF (25 mg-50 mg/Kg pc) prima della somm-

nistrazione di una dose subletale di Fe-NTA (15 mg Fe/Kg pc). Il trattamento con il Fe-NTA ha indotto una significativa riduzione della concentrazione degli UFA e del colesterolo, insieme ad un aumento degli acidi grassi idroperossidi (HP) e del 7-chetocolesterolo (7-cheto). La somministrazione i.p. della PF ha significativamente inibito la degradazione ossidativa degli UFA e del colesterolo, riducendo la formazione di HP e 7-cheto. I composti fenolici possono legarsi alle particelle lipoproteiche aumentando la loro resistenza all'ossidazione o possono agire sequestrando i radicali presenti nell'ambiente acquoso del compartimento plasmatico.

Introduction

In recent years, the interest of scientists has been focused on the preventive effects of olive oil phenols against reactive oxygen species mediated degenerative diseases. It has been reported that phenolic compounds are able to interact with biological systems and to act as bioactive molecules; in particular they are important inhibitors of lipid peroxidation (1), and are believed to be effective through their free radical scavenging and metal chelating properties (2, 3). Lipid peroxidation is regarded as one of the basic mechanism of tissue damage by free radicals.

An excellent model of *in vivo* free radical induced damage, associated with extensive peroxidation of membrane lipids is the ferric-nitrilotriacetate (Fe-NTA) model (4, 5). Intraperitoneal injection (i.p.) of the iron-complex causes renal and hepatic damage by increasing oxidative stress (6, 7). Fe-NTA crosses the mesothelium after i.p. injection and is absorbed through the portal vein into the liver and general circulation (8). A small fraction reaches the kidneys, where the low molecular weight complex is easily filtered through the glomeruli and concentrated in the proximal tubules, where Fe^{3+}-NTA is reduced to Fe^{2+}-NTA (9); the auto-oxidation of Fe^{2+}-NTA generates superoxide radicals, which subsequently potentiate the iron-catalyzed Haber-Weiss reaction to produce hydroxyl radicals, that attack the proximal tubular epithelia from the luminal side (10, 11). Kidney is reported to be the main target of Fe-NTA oxidizing action, while the liver is involved to a less extent; in particular, Fe-NTA oxidative damage starts from the plasma compartment, where Fe-NTA finds the ideal environment to react with oxidizable lipids and proceeds with a similar pattern but a different speed and severity in the kidney and liver (12). The lipid fraction is the first target of the oxidative process and modification of the profile of the major oxidizable lipids in the cell membranes and lipoprotein particles, fatty acids and cholesterol, in the rats treated with Fe-NTA, has been reported as sensible marker of the ongoing oxidative process (12-14).

We investigated the effects exerted by the phenolic fraction (PF) of olive oil against the oxidative lipid damage in the plasma of rats exposed to acute dose of Fe-NTA. Literature data on olive oil polyphenols mainly concern purified compounds, while the antioxidant properties of the total PF has been poorly investigated. Being a complex mixture of compounds, the measure of the total antioxidant capacity could be more representative than the protective effect of a single component.

Materials and methods

All solvents used were HPLC grade (Merck, Darmstadt, Germany); all

other reagents and chemicals were purchased from Sigma Chemical (St. Louis, MO) or CIBA-Geigy (Basel, Switzerland) and were of the highest available purity. The Fe-NTA solution was prepared immediately prior to use as previously described (8). The phenolic extract was prepared from virgin olive oil by a standard procedure and subsequently analysed by HPLC (15).

Animals and treatment

Adult male Wistar rats were purchased from Charles River Italy (Calco, Italy). The rats were housed in solid bottom polycarbonate cages with wire tops in a room maintained at 22±2°C and fed a non-purified diet and tap water ad lib. Six animals/groups (bw 180-200 g) were used for each trial after one week of acclimatization. Rats treated with the PF (n=6) were administered i.p. 25 or 50 mg/Kg bw in 0.9% NaCl solution containing 3% ethanol and 10% Tween 80 (vehicle) or vehicle. The PF was dissolved immediately prior to use. After 30 minutes, animals were injected i.p. with either physiological saline or Fe-NTA solution (15 mg Fe/Kg bw) and after 1 h were deeply anaesthetized and heparinized blood was collected. The blood was immediately centrifuged at 4°C for 10 min at 500 x g to obtain plasma, stored at −80°C for subsequent biochemical analyses.

Lipid extraction and preparation of fatty acids and cholesterol

Total lipids were extracted from an aliquot of 0.7 ml of plasma by the Folch et al. (16) procedure and quantified by the method of Chiang et al. (17). Separation of cholesterol and free fatty acids was obtained by mild saponification as previously described (12).

HPLC analyses

Separation of UFA and cholesterol was carried out with a Agilent Technologies (Palo Alto, CA) 1100 liquid chromatograph equipped with a diode array detector. Cholesterol, detected at 203 nm, and 7-keto, detected at 245 nm, were measured using a Varian column (Middelburg, The Netherlands), Inertsil 5 ODS-3, 150 x 3 mm, and MeOH as mobile phase, at a flow rate of 0.4 ml/min. UFA, detected at 200, and HP, detected at 234 nm, were measured using a Varian column, Inertsil 5 ODS-2, 150 x 4.6 mm, with a mobile phase of CH_3CN/H_2O (70/30, v/v) containing CH_3COOH 0.12% at a flow rate of 1.5 ml/min.
The identification of the peaks was made using standard compounds and second derivative as well as conventional UV spectra, generated using the Agilent Chemstation A.10.02. software.

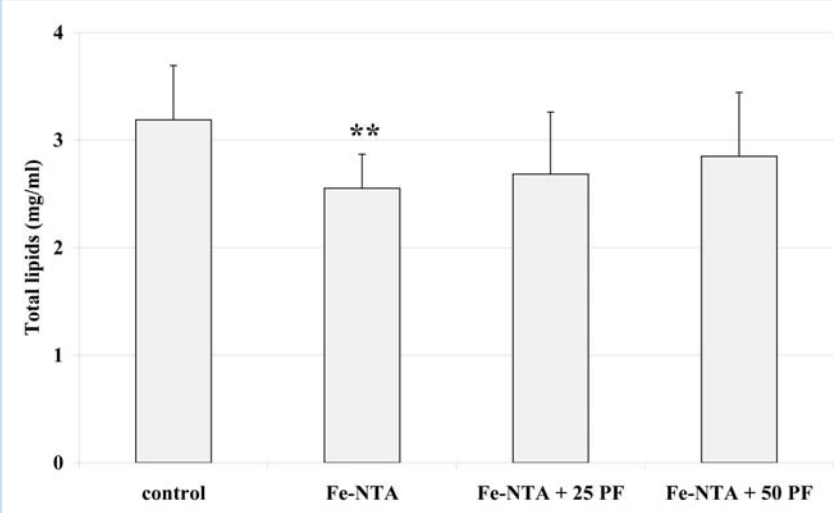
Statistical analysis

INSTAT software (GraphPad software, San Diego, CA) was used to calculate the means and standard deviations of three independent experiments (n=18 for each sample/condition). One-way ANOVA was used to test whether the group means differed significantly.

Results and discussion

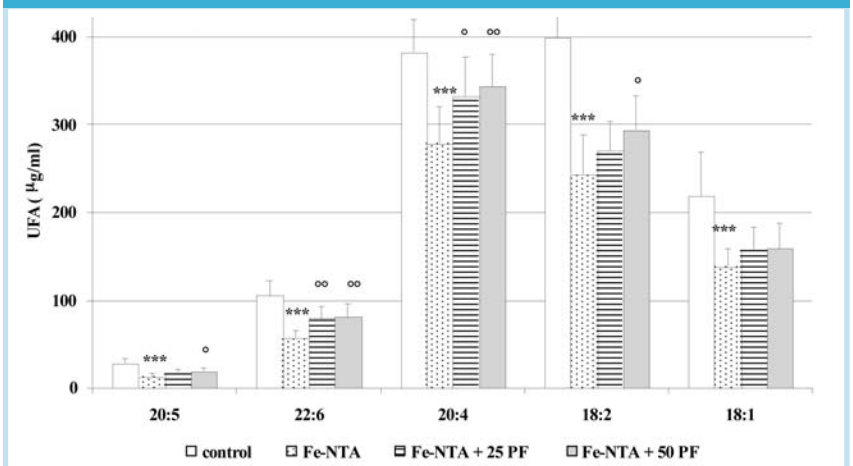
Recent studies demonstrate that olive oil polyphenols are powerful antioxidants in vitro and possess other biological activities that could account for their beneficial health effects (18). However, despite the large body of evidence concerning the beneficial effect of olive oil polyphenols, only a limited number of reports have indicated that these compounds directly suppress oxidative damage *in vivo*. The Fenton chemistry Fe-NTA model is a unique model for studying the ability of antioxidant compound to afford protection against oxidative damage *in vivo*, in particular against the lipid peroxidation involved in tissue damage (19, 20). The modification of the profile of the major oxidizable lipids of cell membranes and lipoproteins, fatty acids and cholesterol, in the rats treated with Fe-NTA, has been reported as sensible marker of the ongoing oxidative process (12-14). To evaluate the ef-

Figura 1 - Concentration of total lipids measured in plasma after i.p. injection of Fe-NTA in the non-treated and treated animals with the phenolic fraction (PF). ** = p<0.01 versus controls



fect of the PF against oxidative stress, rats were treated with the extract (25 mg or 50 mg/Kg bw) prior to Fe-NTA administration.

Figura 2 - Concentration of unsaturated fatty acids (UFA), 20:5 eicosapentaenoic acid, 22:6 docosahexaenoic acid, 20:4 arachidonic acid, 18:2 linoleic acid and 18:1 oleic acid, measured in plasma after i.p. injection of Fe-NTA in the non-treated and treated animals with the phenolic fraction (PF). *** = p<0.001 versus controls; °° = p<0.01; ° = p<0.05 versus Fe-NTA treated animals



I.p. injection of a sub-lethal dose of Fe-NTA, together with kidney and liver oxidative damage, leads to a severe oxidative process in the plasma lipid fraction of treated rats.
Total lipids were extracted from the plasma compartment, where lipid peroxidation is evident at 1h after Fe-NTA treatment (12); as reported in figure 1, the concentration of the total lipids showed a significant decrease, around 20% of the initial value, in the plasma of the treated rats. No significant protection was observed in the rats treated with the PF. The profile of the major UFA was also assessed. Fe-NTA treatment resulted in a strong and extremely significant decrease of the main UFA level in the plasma compartment, as shown in figure 2. Reductions ranged between 50% for 20:5 and 30% for 20:4. PF supplementation, prior to i.p. injection of Fe-NTA, significantly protected the measured UFA against lipid peroxidation from the dose of 25 mg/Kg bw. Fatty acids main oxidation products, fatty acids hydroperoxides (HP), were also detected at 1h after Fe-NTA treatment and obtained results are reported in figure 3. A steep and significant increase of the concentration of HP, three times the initial value, inversely correlated with the depletion of the UFA concentration, expressed as total UFA (Fig. 3), was observed in the Fe-NTA treated animals, indicating an ongoing oxida-

Figura 3 - Concentration of total unsaturated fatty acids (UFA tot) and fatty acids hydroperoxides (HP) measured in plasma after i.p. injection of Fe-NTA in the non-treated and treated animals with the phenolic fraction (PF). *** = p<0.001 versus controls; °°°= p<0.001; ° = p<0.05 versus Fe-NTA treated animals

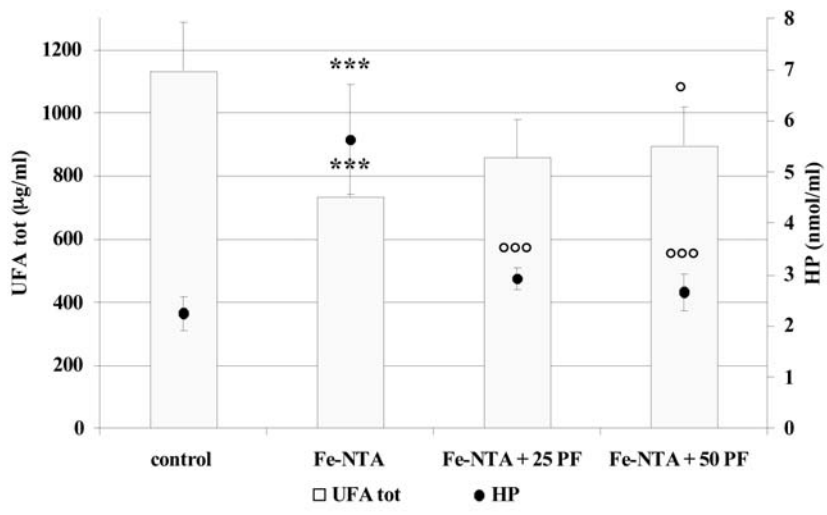
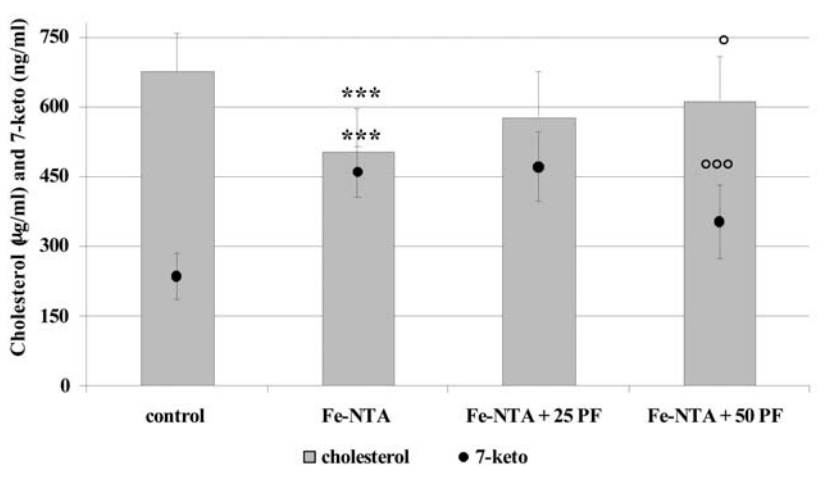


Figura 4 - Concentration of cholesterol and 7-ketocholesterol (7-keto) measured in plasma after i.p. injection of Fe-NTA in the non-treated and treated animals with the phenolic fraction (PF). *** = p<0.001 versus controls; °°°= p<0.001; ° = p<0.05 ; versus Fe-NTA treated animals



tive process. Pretreatment with the PF inhibited completely the increase of HP from the dose of 25 mg/Kg bw. Figure 4 shows the concentration of cholesterol and its major oxidation product 7-keto, measured in the plasma of control and treated rats. Cholesterol level was significantly decreased at 1h after injection, with a reduction of 30%, and a related increase of 7-keto, twice the initial value, was also observed. Supplementation with the extract, 50 mg/Kg bw, significantly protected cholesterol from oxidation: the loss of cholesterol was 10% of the initial value and the concentration of 7-keto was significantly lower.

Our data are the first evidence showing the protective effect of the total phenolic fraction of olive oil against oxidative damage *in vivo*: phenolic compounds systemically available significantly preserved the concentration of the main plasma lipids, UFA and cholesterol, and thus reduced the oxidative modification of the lipoprotein particles. The ability of the PF to inhibit lipid peroxidation in the lipoproteins is consistent with a wide number of *in vitro* studies and the observation *ex vivo* of Leenen et al. (21). The phenolic compounds present in the plasma compartment, because of their relatively nonpolar nature, may associate with lipoproteins particles (22, 23), increasing their resistance against oxidation or may directly trap radi-

cals generated in the plasma aqueous compartment (18). The mechanism by which the PF exerts its protection in the Fe-NTA model is complex and need further investigation, however it could be postulated to be mainly due to the scavenging and chelating abilities of its components. This investigation adds to the body of literature showing how consumption of high-quality, phenol-rich olive oil provides healthful compounds whose biological activities might contribute to prevention of degenerative diseases.

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