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Antioxidant activity of phenolic acids and esters present in red wine on human low-density lipoproteins

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RÉSUMÉ

Trois expériences ont été menées afin d'évaluer l'activité antioxydante de différents acides et de leurs esters. Une analyse quantitative par résonance paramagnétique électronique (RPE) a été réalisée en utilisant le système acétaldéhyde/xanthine oxydase et la réaction de Fenton générant, respectivement, les radicaux superoxyde et hydroxyle. Dans un second test, les hydroperoxydes générés par une réaction d'oxydation des lipoprotéines de basse densité (LDL) catalysée par Cu^{2+} ont été quantifiés par une méthode iodométrique modifiée. Dans une troisième étude, les LDL ont été oxydées par la méthode d'Esterbauer et les espèces oxydées ont été quantifiées par HPLC. Les résultats montrent que les dérivés estérifiés présentent une activité antioxydante contre la lipoperoxydation des LDL bien plus importante que celle des acides phénoliques correspondants.

Mots-clé : antioxydants, composés phénoliques, lipoprotéines, vin rouge.

ABSTRACT

To evaluate the antioxidant activity of different phenolic acids and their esters, three types of experiments have been used. Electron paramagnetic resonance (EPR) quantitative analysis was carried out using the acetaldehyde/xanthine oxidase system and Fenton's reaction to generate superoxide and hydroxyl radicals, respectively. In a second test, hydroperoxides generated by Cu^{2+} -catalysed oxidation of low density lipoproteins (LDL) were quantified by a modified iodometric method. In a third assay, LDL were oxidized with Esterbauer's method and modified LDL species were quantified by HPLC. The results show that the esterified phenolic derivatives present a better antioxidant activity, on the lipoperoxydation of LDL, than the corresponding phenolic acids.

Key words : antioxidants, phenolic compounds, lipoproteins, red wine.

INTRODUCTION

Several lines of evidence implicate oxidatively damaged LDL as atherogenic agents. By activating inflammatory events, oxidized lipoproteins may contribute to all stages of the atherosclerotic process [1]. Because atherosclerotic vascular disease is the leading cause of death among persons with a western life-style, there has been much interest in recent years in the possible effects of antioxidants in retarding oxidative modifications of LDL. The "French Paradox", i.e. a relatively low incidence of cardiovascular events in spite of diets high in saturated fat, is attributed to the regular drinking of red wine [2]. Since most of the phenolic acids contained in wine are present as ester derivatives, the aim of our work was to compare the antioxidant capacity of both chemical forms. Three tests were carried out, one using the EPR method and spin trapping technique, the second measuring hydroperoxides after the Cu^{2+} -catalysed oxidation of LDL and the third based on the determination of oxidized LDL by HPLC.

MATERIAL AND METHODS

Reagents

Xanthine oxidase, 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) used as spin trap were obtained from Sigma Chemical Co.; gallic, caffeic, ferulic and sinapic acids were from Aldrich Chemical Co.; acetaldehyde, ethylenediaminetetraacetic acid (EDTA), H_2O_2 were from Prolabo. DMPO was purified and prepared as reported elsewhere [3]. Synthesis of ethanolic esters was performed as previously described [4].

In vitro EPR tests

Hydroxyl radical scavenging tests

A 1 ml solution was prepared in a glass tube by adding the reagents in the following order : DMPO (50 mM), H_2O_2 (100 mM), EDTA (104 μM), phenolic compound (10^{-4}M), FeSO_4 (100 μM). For more details see [5].

Superoxide radical scavenging tests

A 1 ml solution was prepared in a glass tube by adding the reagents in the following order : DMPO (50 mM), xanthine oxidase (18 mU/mL), phenolic compounds (10^{-4} M) and acetaldehyde (60 mM) in phosphate buffer (pH 7.4). For more details see [6].

LDL peroxidation tests

Native LDL were isolated from human plasma [7]. The effects of phenolic compounds on the susceptibility of LDL to oxidation were investigated by determining the hydroperoxide concentration after the Cu^{++} -catalysed oxidation of LDL. A kinetic adaptation of El Saadani's iodometric method was used [8].

LDL oxidation tests

LDL were oxidized with Esterbauer's method [9] by exposure to 1.6 μM copper ions (Cu^{++}) in phosphate buffer for 3-8 h at 25°C. This procedure generate three LDL species, then separated by HPLC [10].

RESULTS AND DISCUSSION

In order to estimate the antioxidant activity of phenolic acids and their esters, three methods were used. For the EPR studies the control signals were obtained from the Fenton or acetaldehyde/xanthine oxidase reaction with DMPO as spin trap. The % inhibition of the intensity of the HO^{\cdot} or $\text{O}_2^{\cdot-}$ spin adduct signals were determined after adding, successively, gallic acid, caffeic acid, ferulic acid, sinapic acid, ethyl gallate, ethyl caffeate, ethyl ferulate and ethyl sinapate (10^{-4} M) (Fig. 1a, 1b). Considering the HO^{\cdot} test, ethyl ferulate presents a higher antioxidant capacity than the other compounds tested. When the $\text{O}_2^{\cdot-}$ test was carried out at a 10^{-4} M concentration of the tested compound, caffeic acid or ethyl caffeate led to the same % inhibition of the EPR signal (100%, Fig. 1b). When the concentration of these two tested compounds was decreased to 10^{-5} M, the % inhibition of the EPR signal was still 100% for ethyl caffeate and

61±5% for caffeic acid. Taking into account both tests, an eight tested compounds, except ferulic acid, presented a higher scavenging activity against O₂⁻ than against HO[•].

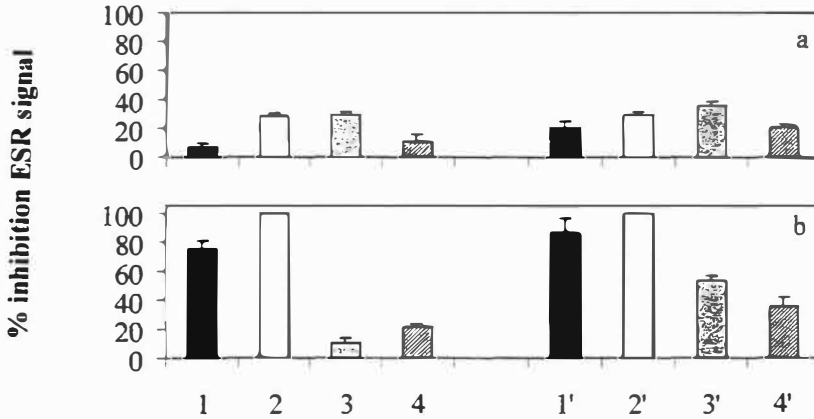


Figure 1 : Antioxydant activity of phenolic acids and esters determined by the inhibition of the signal intensity of hydroxyl (a) and superoxide (b) DMPO-adduct EPR signals with gallic acid (1), caffeic acid (2), ferulic acid (3), sinapic acid (4), ethyl gallate (1'), ethyl caffeate (2'), ethyl ferulate (3') and ethyl sinapate (4').

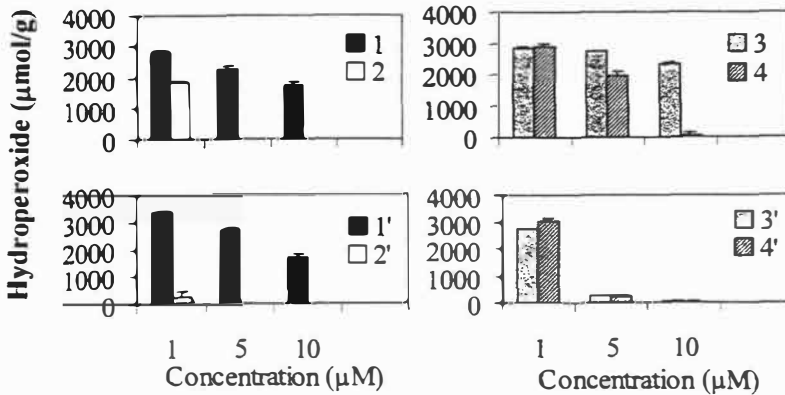


Figure 2 : Concentration of hydroperoxides after LDL oxidation as a function of the phenolic compound added (gallic acid (1), caffeic acid (2), ferulic acid (3), sinapic acid (4), ethyl gallate (1'), ethyl caffeate (2'), ethyl ferulate (3') and ethyl sinapate (4')).

In a second test, the protective effect of phenolic acids and esters on the susceptibility of LDL to oxidation, was determined by measuring hydroperoxides formed by copper-mediated oxidation using the iodometric method [8].

At a concentration of 10 μM , caffeic and sinapic acids, their esters and ferulic ester delayed the LDL peroxidation (Fig. 2).

In a third assay, LDL were oxidized with Esterbauer's method. The Apo B oxidation increased the negative charge of the protein leading to three LDL fractions A, B, C, which can be separated by HPLC using an anion exchange column. The phenolic esters (10 μM) presented a better protective effect against oxidation than the corresponding acids, except ethyl gallate (Fig. 3).

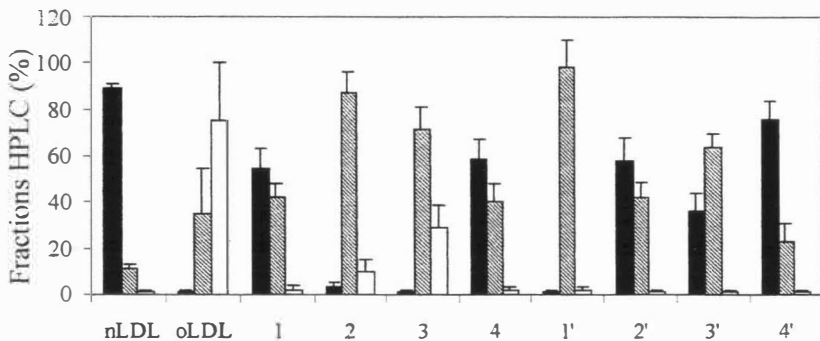


Figure 3 : Pourcentage of the different LDL HPLC fractions (A : ■, B : ▨, C : □) as a function of the phenolic compound added (gallic acid (1), caffeic acid (2), ferulic acid (3), sinapic acid (4), ethyl gallate (1'), ethyl caffeate (2'), ethyl ferulate (3') and ethyl sinapate (4'), native LDL (nLDL), oxidized LDL (oLDL)).

These observations are consistent with the EPR tests (Fig. 1). However, in this third test, the sinapic acid prevents LDL from oxidation although no effect was observed with the EPR analysis.

In conclusion, phenolic esters appeared more active than their corresponding acid forms in our experimental conditions. Ethyl caffeate and ethyl ferulate presented the highest antioxidant activities.

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