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Antioxidant properties of resveratrol and piceid on lipid peroxidation in micelles and monolamellar liposomes

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ABSTRACT

The antioxidant activities of *trans*-resveratrol (*trans*-3,5,4′-trihydroxystilbene) and *trans*-piceid (*trans*-5,4′-dihydroxystilbene-3-*O*-β-D-glucopyranoside), its more widespread glycosilate derivative, have been compared measuring their inhibitory action on peroxidation of linoleic acid (LA) and the radical scavenging ability towards different free radicals (such as DPPH) and radical initiators. It has been found that the two stilbenes have similar antioxidant capacity, while the comparison with BHT (2,6-di-*tert*-butyl-4-methylphenol) and α-tocopherol (vitamin E, vit. E), taken as reference, points out a slower but prolonged protective action against lipid peroxidation. Furthermore, piceid appears more efficacious than resveratrol as a consequence of the reaction of the latter with its radical form.
The DSC profiles of phosphatidylcholine liposomes of various chain lengths, and EPR measurements of spin labelled liposomes demonstrated that the susceptible hydroxyl group of these compounds are located in the lipid region of the bilayer close to the double bonds of polyunsatured fatty acids, making these stilbenes particularly suitable for the prevention and control of the lipid peroxidation of the membranes.

1. Introduction

It is known that a diet rich in fruit and vegetables helps to prevent cardiovascular diseases, cancer, and ageing-related disorders [1].

The effect can be traced back to the antioxidant activity of polyphenols of fruits and vegetables, able to inhibit lipid peroxidation, forced by free radicals, and related to chronic health problems like cancer, arthrosclerosis and ageing [2–4]. In particular resveratrol, a polyphenol mainly present in grapes and red wine, demonstrated interesting biomedical properties for its inhibitory effects on cancer promotion and propagation [5], cardioprotective action due to inhibition of the oxidation of low-density lipoprotein (LDL) [6] and of platelet aggregation [7,8], anti-inflammatory activities and preventive effect on Alzheimer's disease and dementia [9].

Recently it was found that, in the grape juices, the average concentration of piceid, the glycoside form of resveratrol, is seven times that of resveratrol [10] and this is probably the most abundant form of resveratrol in nature [11].

A number of studies hypothesise that piceid may have biomedical properties similar to those above mentioned for resveratrol: anticarcinogenic effects [12,13] and inhibition of platelet aggregation [14,15] and of oxidation of LDL [16].

The aim of this work is to understand if, by analogy, piceid has also antioxidant properties comparable to resveratrol.

Unfortunately the antioxidant capacity of a compound and its effectiveness in preventing diseases cannot be unequivocally determined. Roughly the experimental methods can be grouped into two classes, based on hydrogen atom transfer (HAT) and on single electron transfer (ET) [17]. Therefore, we studied the antioxidant activity of resveratrol and piceid in terms of their inhibitory action on peroxidation of linoleic acid (LA), an HAT assay, and as radical scavenging ability towards 2,2′-diphenyl-1-picrylhydrazyl (DPPH method), an ET assay. The scavenging reaction of resveratrol is accompanied by the formation of a product which is not formed in the analogue reaction of piceid, and the data support the hypothesis of a reaction between one molecule of resveratrol and one molecule of its radical. This point, the possibility that an antioxidant may react with itself, is of general interest and must be accounted for in evaluating the antioxidant activity of a molecule.

At membrane level there is an assumed relationship between the rate of lipid peroxidation and membrane composition and fluidity [18–20], implying that the efficacy of an antioxidant can be meaningfully modulated by its collocation inside the bilayer. From this point of view, liposomes are much more representative models of biological membranes than micelles, so the peroxidation measurements were repeated in monolamellar liposomes. Contemporarily the interactions of resveratrol and piceid with multilamellar liposomes of satured L-α-phosphatidylcholine (PC) of various chain lengths (dimyristoyl, DMPC, dipalmitoyl, DPPC, distearoyl, DSPC) were investigated, determining

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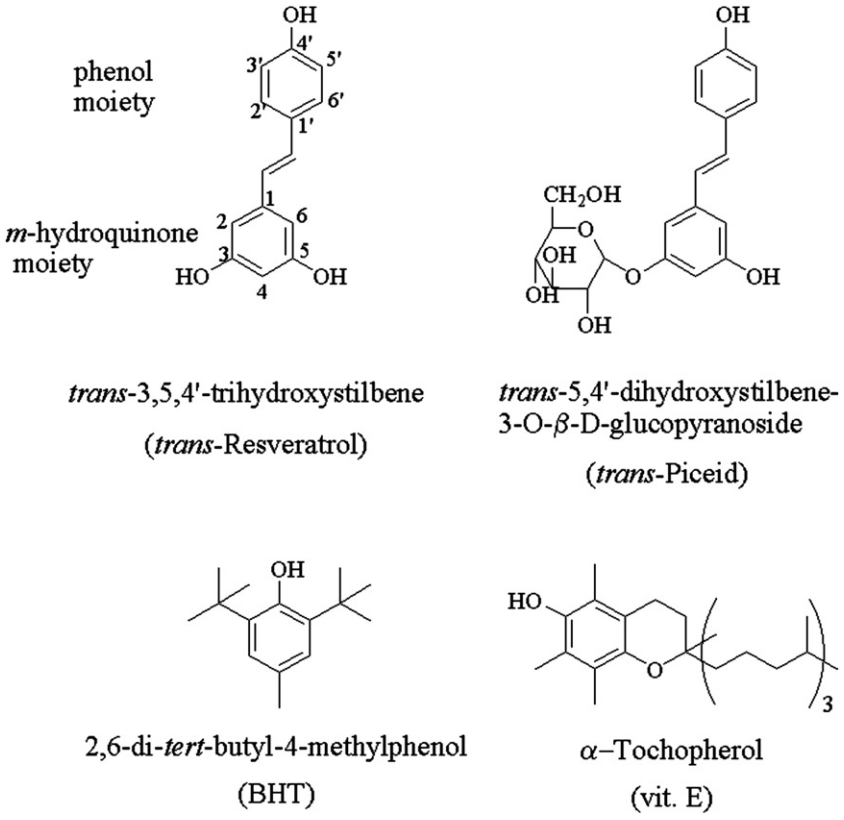

Fig. 1. Molecular structure of studied compounds.

the partition coefficients of the drugs, the decay of the ordered lipid organisation they produce, and their position in membranes using differential scanning calorimetry (DSC) and spin labelling EPR techniques.

Data point out how both these compounds are located in the lipid region of bilayer close to double bounds of LA and have, on average, a slower but longer antioxidant capacity than BHT and α-tocopherol taken as reference (Fig. 1).

The comparison between micelles and monolamellar liposomes demonstrated as well that the inhibitory effect of vit. E, measured in liposomes, is about one half the same effect when measured in micelles. This will be explained in terms of a different accessibility of the phenolic groups of vit. E to the double bonds of unsaturated lipid chains, and demonstrated that the true antioxidant power of vit. E could be overestimated by usual measurements in micelles.

2. Materials and methods

2.1. Chemicals

All chemicals, of the highest available quality, were obtained from Sigma Chemical Co. (St. Louis, USA). ABIP was a kind gift of Wako Chemicals USA. *Trans*-piceid and *trans*-resveratrol, with a purity grade higher than 99%, have been supplied by the Istituto Agrario di S. Michele all'Adige (IT) [21]. The aqueous solutions were prepared with quality milliQ water.

2.2. Inhibition of lipid peroxidation

The antioxidant activity of resveratrol and piceid to prevent LA peroxidation was studied in sodium dodecyl sulfate (SDS) micelles and in monolamellar liposomes of DPPC.

The peroxidation was initiated by the thermolabile water-soluble azo initiator 2,2′-azobis[2-(2-imidazolin-2-yl)propane] (ABIP) and measured as rate of oxygen consumption. The reaction was carried on in a closed thermostated vessel (37 °C) and the oxygen consumption was followed using a Clark electrode.

In SDS micelles (50 mM) the concentration of LA was 10 mM, while in DPPC monolamellar liposomes (20 mM) LA was 2.5 mM; the choice of the concentrations was the result of a series of tests at different concentrations and represents the best compromise between a high rate of peroxidation and a minor change in lipid organization due to LA [22]; in both cases we used phosphate buffer (50 mM, pH 7.4).

To the solution, previously equilibrated with air and kept at constant temperature (37 °C), the azo initiator ABIP (4 mM final concentration) was injected; and the oxygen consumption was monitored. After a few minutes an ethanol solution of antioxidant was added ($2\cdot10^{-6}$ M final concentration). Every experiment was repeated three times.

The antioxidant capacity was calculated as change of slope of oxygen consumption before and after the addition of the antioxidant; in particular it is expressed by the percentage inhibition of the peroxidation [23]:

$$P_{inh}\% = \frac{R_p - R_{inh}}{R_p}\cdot 100$$

Where R_p is the rate of propagation of lipid peroxidation due to ABIP and R_{inh} is the rate of inhibition of peroxidation after the addition of antioxidant.

2.3. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay

This method is based on the capacity of an antioxidant to scavenge the stable free radical DPPH [24]. In the reaction DPPH goes through a change of color so its disappearance can be monitored spectrophotometrically at 515 nm (ε=10,800 M^{-1} cm^{-1}) [25]. The reaction was carried on at 25 °C in ethanol at various antioxidant concentrations (12.5, 25, 50 μM), while that of DPPH was fixed at 100 μM.