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Peroxynitrite Scavenging by Wines

Keywords: *Antioxidant; flavonoid; polyphenol; peroxynitrite; wine*

INTRODUCTION

The antioxidant activity of wine is intensively investigated. This is partly due to the French paradox, the apparent compatibility of high fat consumption with a low incidence of coronary heart disease (CHD). This compatibility has been ascribed to the relatively high intake of wine in France (Frankel et al., 1993). The antioxidant activity of wine is thought to be a major cause of the CHD-preventing effect. Known antioxidant effects of wine are the scavenging of free radicals and the inhibition of radical-producing enzymes (Facino et al., 1994; Ricardo da Silva et al., 1991; Sato et al., 1996).

It has recently been reported that flavonoids in wine effectively scavenge nitric oxide (Verhagen et al., 1996) and in this way wine ameliorates nitric oxide induced tissue damage. Nitric oxide toxicity is for the greater part mediated by peroxynitrite ($ONOO^-$), formed in the reaction of nitric oxide with superoxide radicals (Rubbo et al., 1996). Various toxic effects of peroxynitrite have been described. For example, peroxynitrite oxidizes low-density lipoprotein (LDL) (Graham et al., 1993), a key process in the etiology of atherosclerosis. To further evaluate the possible protection provided by wine against NO toxicity, the ability of wine to scavenge peroxynitrite was determined.

MATERIALS AND METHODS

All of the wines were from the same origin as previously used to determine the NO scavenging of wines (Verhagen et al., 1996). The brand used was Torres (Penedès, Spain); the red wine was called Sangre de Toro 1994 (Garnacha and Carineña grapes), the rosé was named De Casta Rosado 1993 (Garnacha and Carineña grapes), and the white wine was Gran Viña Sol 1993 Chardonnay Penedès-denominción de Origen (85% Chardonnay and 15% Parellada grapes).

Peroxynitrite scavenging was measured by the oxidation of dihydrorhodamine 123, as described by Kooy et al. (1994). Fluorescence measurements were performed on a Shimadzu RF-5001 PC fluorometer with excitation and emission wavelengths of 500 and 536 nm, respectively, at 37 °C. The effects are expressed as the concentration giving 50% inhibition of the oxidation of dihydrorhodamine 123 (IC_{50}).

Polyvinylpolypyrrolidone (PVPP Divergan W) treatment to indiscriminately remove the polyphenols from the red wine was performed according to the method of Glenn et al. (1972).

The polyphenol index of the wines was determined as the absorption of wine at 280 nm, if necessary in a diluted sample. Absorptions were measured on a Pharmacia Biotech Ultrospec 2000.

RESULTS AND DISCUSSION

All of the wines tested showed a good peroxynitrite scavenging activity. Red wine is an especially excellent scavenger of peroxynitrite, 10 times better than rosé and 18 times better than white wine (Table 1). Removing the polyphenols from red wine by PVPP treatment drastically diminished the peroxynitrite scavenging activity: the activity was reduced 185-fold. Moreover, the peroxynitrite scavenging activity of the wines correlated nicely with the polyphenol index of the wines (Figure 1). These findings again indicate that the polyphenols are the most important antioxidants in wine.

To evaluate the peroxynitrite scavenging activity of wine, the activity of wine is compared to that of ebselen. Ebselen is a selenium-containing compound that displays a glutathione peroxidase activity (Haenen et al., 1990). Recently, it has been reported to be an excellent peroxynitrite scavenger (Masumoto, 1996). Ebselen is much more active than methionine and cysteine. On the basis of the results obtained with ebselen, it has been speculated that selenium-containing proteins—with so far unknown functions—form the endogenous line of defense against peroxynitrite (Masumoto, 1996).

In our assay ebselen has an IC_{50} of 1.99 μM. It can be calculated that red wine has the same peroxynitrite scavenging capacity as a 20 mM solution of ebselen, and one glass of wine (100 mL) equals as much as 0.5 g of ebselen. This illustrates the very potent peroxynitrite

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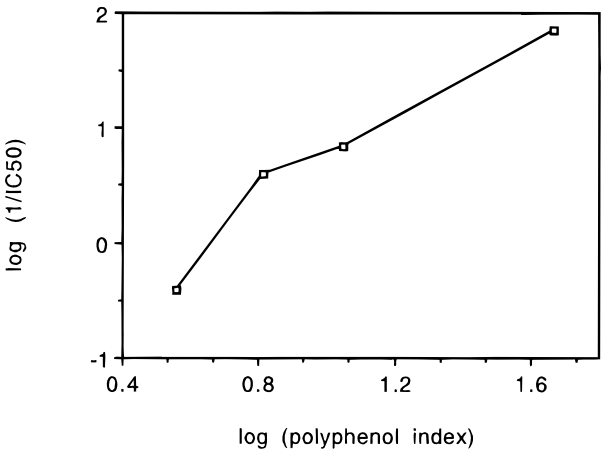

Figure 1. Correlation between the polyphenol index and peroxynitrite scavenging activity. The correlation coefficient between the polyphenol index and $1/IC_{50}$ determined using linear regression was 0.97.

Table 1. IC_{50} in Peroxynitrite Scavenging (Mean ± SD, *n* = 6) and the Polyphenol Index of the Wines Tested

wine	IC_{50} peroxynitrite scavenging (%, v/v)	polyphenol index
red wine	0.014 ± 0.003	46.2
rosé	0.145 ± 0.011	11.2
white wine	0.251 ± 0.022	6.52
PVPP-treated red wine	2.58 ± 0.17	3.60

scavenging ability of wine. On the basis of these results, it is tempting to conclude that flavonoids, rather than selenium-containing proteins, form the major line of defense against peroxynitrite. One of the major sources of flavonoids is wine. Of course, further evidence is needed to fully substantiate this hypothesis.

As reported previously, the polyphenols in wine are able to scavenge superoxide anion radicals (Sato et al., 1996) and the nitric oxide radical (Verhagen et al., 1996), the two ingredients needed to form peroxynitrite. In the present study it is shown that the polyphenols in wine also scavenge peroxynitrite very efficiently. This nicely coincides with the reported peroxynitrite scavenging of epigallocatechin gallate, a polyphenolic tea antioxidant (Fiala et al., 1996). Extensive peroxynitrite production in atherosclerotic lesions has been demonstrated (Beckman et al. 1994). In addition, peroxynitrite is known to oxidize LDL (Graham et al., 1993). LDL oxidation is known to occur at an early stage in the etiology of atherosclerosis (Steinberg et al., 1989). The potent scavenging of peroxynitrite by the polyphenols in red wine may prevent LDL oxidation, which may finally lower the incidence of CHD. Also, other processes in the etiology of CHD in which peroxynitrite toxicity plays a role can be inhibited by wine. However, one should keep in mind that data on the absorption, metabolism, distribution, and excretion of the flavonoids in wine are scrace.

In conclusion, the finding that wine, especially red wine, very efficiently scavenges peroxynitrite adds a new aspect to the antioxidant profile of wine.

ABBREVIATIONS USED

CHD, coronary heart disease; PVPP, polyvinylpolypyrrolidone; LDL, low-density lipoprotein; IC_{50}, concentration of wine (expressed in %, v/v) or ebselen (expressed in μM) that gives 50% inhibition.

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