Nitric Oxide Radical Scavenging by Wines

Keywords: *Nitric oxide radical (•NO); scavenging; wine; polyphenols; flavonoids*

INTRODUCTION

The French paradox, the apparent compatibility of a high-fat diet with a low incidence of CHD, has been ascribed to the relatively high consumption of wine in France (Frankel et al., 1993). Although the exact mechanism has not been elucidated, the antioxidant activity of wine is thought to be the major contributor to its CHD-preventing effect. Various antioxidant effects of wines have been demonstrated, e.g. scavening of radicals such as superoxide radicals and inhibition of radical producing enzymes (Facion et al., 1994; Ricardo da Silva et al., 1991; Sato et al., 1996). To further characterize the antioxidant potential of wine, we tested the total antioxidant capacity of three well-defined wines. Recently, the potent nitric oxide radical (•NO) scavenging activity of flavonoids has been described (Van Acker et al., 1995). Since wine has a high content of flavonoids, the •NO scavenging potency of wine was assessed.

MATERIALS AND METHODS

The wines used were all of the brand "Torres" (Penedès, Spain): the red wine was named "Sangre de Torro 1992" (Garnacha and Cariñera grapes), the rosé "De Casta Rosado 1993" (also Garnacha and Cariñena), and the white "Gran Viña Sol 1993 Chardonnay Penedès-denominción de Origen" (85% Chardonnay and 15% Parellada).

The total antioxidant capacity was determined according to the method of Rice-Evans et al. (1995). The antioxidant capacity reflects the number of radicals that can be scavenged. The resulting value is called the TEAC: the antioxidant capacity of a compound relative to that of Trolox (a water-soluble α-tocopherol analog). The TEAC value of the wines is given as the concentration of a Trolox solution (in millimolar) that has an equal radical scavenging capacity as the undiluted wine sample. The TEAC value of α-tocopherol given in the text is the relative scavenging capacity of this compound compared to that of Trolox on a molar basis.

The •NO scavenging ability of wines was determined as described by Vriesman et al. (1996). The rate constant (k_s) of the second-order reaction between the scavenger and •NO was calculated. Corrections for the spontaneous degradation of •NO were made. PVPP (Divergan W) treatment according to the method of Glenn et al. (1972) was performed to remove polyphenols from red wine.

The polyphenol index is determined as the absorption of wine at 280 nm, if necessary in a diluted sample.

RESULTS AND DISCUSSION

The results of the wines tested show that the red wine has a TEAC of 14.1 mM, which is about 6 times higher than that of the rosés (2.41 mM) and about 17 times the TEAC of the white wine (0.82 mM) (Table 1). The TEAC of the different wines is well within the range reported earlier for these types of wine (Campos and Lissi, 1996). As α-tocopherol has a TEAC of 0.97 ± 0.01, it can be calculated that one glass (100 mL) of red wine equals 63 times the antioxidant capacity provided by the U.S. recommended daily intake of vitamin E (10 mg/day for men). This illustrates the high antioxidant capacity of red wine. One has to keep in mind that the antioxidant capacity is only one aspect of the overall antioxidant activity. Besides the antioxidant capacity, other factors, e.g., the reaction rate with free radicals and the distribution of the antioxidant over the aqueous phase and membranes, are important.

Table 1

sample	TEAC[a] (mM Trolox)	log k_s (min^{-1})
red wine	14.1 ± 0.07	2.95 ± 0.16
red wine (PVPP-treated)	0.30 ± 0.02	0.91 ± 0.32
rosé	2.41 ± 0.03	1.96 ± 0.10
white wine	0.82 ± 0.03	1.32 ± 0.01

[a] Trolox equivalent antioxidant capacity (TEAC) and •NO scavenging capacity of wines. In the experiments the wines had to be diluted due to their potent activity. The results given are extrapolated to the undiluted sample. The TEAC gives the concentration of a solution of Trolox with a similar antioxidant capacity. The •NO scavenging capacity is presented by the pseudo-first-order reaction constant (k_s) of the reaction of •NO in the undiluted sample. The results are presented as mean ± SD (n = 9).

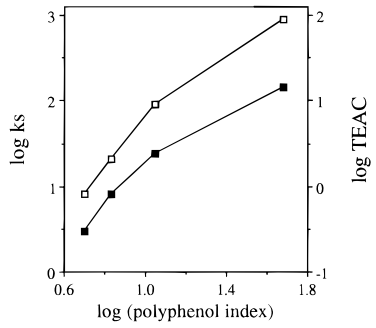

Figure 1. Correlation between the polyphenol index, Trolox equivalent antioxidant capacity (TEAC, solid symbols) and •NO scavenging capacity of wines (k_s, open symbols). The polyphenol indexes for red, rosé, white, and PVPP-treated wine were, respectively, 48.0, 11.1, 6.75, and 5.0. The other data used are presented in Table 1. Using linear regression, the correlation coefficient for both antioxidant parameters with the polyphenol index was 0.98.

Here we also found that red wine is a good scavenger of •NO, 10 times better than rosé and 40 times better than white wine (Table 1). A red wine content of 0.006% in our assay decreases the half-life of •NO by 50%, illustrating its potent •NO scavenging activity. It is known that the different concentrations of phenolic compounds represent the main difference between red wine, rosé, and white wine (Glories, 1988). To examine whether these components were responsible for the differences in antioxidant parameters, polyphenols were removed from red wine by PVPP treatment. PVPP-treated red wine had a 47-fold lower TEAC and a 110-fold lower k_s than untreated red wine. Moreover, it was noticed that the aforementioned antioxidant parameters correlated with the polyphenol index (respectively 48.0, 11.1, 6.75, and 5.0 for red, rosé, white, and PVPP-treated red wine) (Figure 1). Recently, it has been found that also the superoxide radical scavenging potential of wines nicely correlated with the phenolic content of the wines (Sato et al., 1996). These findings again indicate that polyphenols are the major antioxidants in wine, responsible for the total antioxidant capacity and the scavenging of free radicals like superoxide radicals and •NO.

Numerous (patho)physiological processes are influenced by •NO-mediated mechanisms. In principle, all of these processes are prone to be affected by the •NO

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scavenging of wine. It has been found that flavonoids accumulate between the endothelial layer and the vascular smooth muscle cells (Neuman et al., 1992) and, therefore, the main effects of •NO scavenging are expected at this site. Atherosclerosis starts here in a process where undoubtedly •NO plays an unfavorable role. A protective effect of wine in this process is expected. A correlation has been found between the •NO scavenging activity of flavonoids and their ability to protect against vascular endothelial damage (Van Acker et al., 1995). However, data on the bioavailability of the polyphenols are scarce and prooxidant activities have been described. Nevertheless, consumption of red wine increases the TEAC of blood (Maxwell et al., 1994) and an inverse correlation has been found between the intake of flavonoids and the incidence of cardiovascular diseases (Hertog et al., 1993). It is tempting to conclude that, besides the general antioxidant effect, the •NO scavenging activity provided by wines is important in their protection against CHD.

ABBREVIATIONS USED

•NO, nitric oxide radical; CHD, coronary heart disease; PVPP, polyvinylpolypyrrolidone; TEAC, Trolox equivalent antioxidant capacity.

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