RAPID COMMUNICATIONS

Vitamins and Especially Flavonoids in Common Beverages Are Powerful in Vitro Antioxidants Which Enrich Lower Density Lipoproteins and Increase Their Oxidative Resistance after ex Vivo Spiking in Human Plasma

Keywords: *Lipoprotein oxidation; antioxidant; polyphenols; beverages*

INTRODUCTION

Consumption of fruits and vegetables is associated with a lowered risk of cardiovascular disease (Ames et al., 1993). One possible reason for this benefit is the presence of antioxidant vitamins C and E and the provitamin β-carotene in these foods. However, recent supplementation studies with vitamin E and β-carotene have cast doubt on this hypothesis (Rapola et al., 1997). Oxidation of low (LDL) and very low (VLDL) density lipoproteins are crucial steps in atherosclerotic lesion formation (Steinberg et al., 1989). Other dietary components, polyphenolic antioxidants such as flavonoids, have recently been shown in a seven-country epidemiology study to be protective for heart disease (Hertog et al., 1995). Beverages such as red wines and teas are high in polyphenols and provide a large quantity of flavonoids to the Mediterranean and Japanese diets, respectively. The increased consumption of red wines by the French has been used to explain the French Paradox of heart disease (Renaud and de Lorgeril, 1992). Tea drinking was found to reduce the risk of heart disease in a cross-cultural study (Hertog et al., 1995).

We have recently shown that many polyphenols are stronger antioxidants than the vitamin antioxidants using the LDL + VLDL oxidation model (Vinson et al., 1995a). Polyphenols have also been found to bind to these lipoproteins (Vinson and Hontz, 1995) in plasma and protect them from ex vivo oxidation. This lipoprotein-bound antioxidant activity, i.e., enrichment, as measured by our methodology, has been shown to significantly correlate with protein binding (Wang and Goodman, 1999). By binding to lipoproteins, polyphenols can thus provide protection when the lipoproteins penetrate the endothelium of the aorta where they are subsequently oxidized (Steinberg et al., 1989). In this work, we have investigated the quality of the vitamin antioxidants and polyphenolic antioxidants in beverages and their ability to enrich lipoproteins.

MATERIALS AND METHODS

Pure compounds were obtained from Sigma or Aldrich Chemical Co. Beverages were obtained from local supermarkets. Teas and coffees were made fresh from leaves (Wang et al., 1994) and ground coffee (Fried et al., 1992), respectively. LDL + VLDL was isolated from a pool of normolipemic human plasma by affinity column (Vinson and Hontz, 1995). Wine was dealcoholized by low-temperature vacuum distillation. Phenol concentrations in the beverages were measured as catechin equivalents by the Folin−Cocialteu reagent. Beverages containing ascorbic acid were treated with ascorbate oxidase before phenol measurement to remove ascorbate interference with the Folin reaction.

The procedure for determination of the antioxidant concentration to inhibit in vitro LDL + VLDL oxidation 50% (IC_{50}) has been previously published (Vinson et al., 1995a). Ex vivo plasma spiking of pure antioxidants, isolation of the low-density lipoproteins, and measurement of the lag time has been used to compare the effectiveness of lipoprotein-bound antioxidants (Vinson and Hontz, 1995). This is done by measuring the concentration to inhibit the isolated LDL + VLDL oxidation and thus increase the lag time by 50% (CLT_{50}). In this study we also spiked pure antioxidants dissolved in water or methanol or diluted beverages (1−50 μL) at 50−200 μM (catechin equivalents) in the plasma (1 mL). Ubiquinol-10 was formed from coenzyme Q_{10} (ubiquinone) by reduction with sodium dithionite.

RESULTS AND DISCUSSION

The results are shown in Table 1 for pure antioxidant vitamins and nutrients, polyphenols, and beverages. Polyphenols generally were better antioxidants, as measured by IC_{50}, than the vitamins. Among the vitamins and nutrients, ubiquinol-10 was the best antioxidant as was also the case with the LDL model oxidized with the peroxy radical generator AAPH (Thomas et al., 1997).

Teas were found to contain high-quality antioxidants, which is not suprising since epigallocatechin gallate was the most powerful pure antioxidant with an IC_{50} of 0.08 μM (Vinson et al., 1995b) and it is the major polyphenol in green and black tea (Wang et al., 1994). The order of antioxidant quality for the beverages was black tea > coffee > prune juice = beer > green tea > orange juice > red wine > tangerine juice > red grape juice > white grape juice > grapefruit juice.

Ascorbic acid did not exhibit any lipoprotein-bound antioxidant activity. This is not surprising since it is very water soluble and has not been found to enrich lipoproteins after human consumption (Reaven et al., 1993). Tocopherol and retinol, two vitamin phenols, were active in our model and have been reported to bind to LDL in vitro and in vivo after supplementation (Reaven et al., 1993; Livrea et al., 1995). Ubiquinol-10 had good antioxidant properties after enrichment. It has been reported to bind to LDL and to be the first antioxidant in LDL to be oxidized (Stocker et al., 1991). As a result of these antioxidant properties and after absorption of CoQ_{10}, ubiquinol-10 is also an in vivo phenolic antioxidant for LDL oxidation (Mohr et al., 1992). β-Carotene was a very poor in vitro antioxidant but did bind with the low-density lipoproteins LDL +

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Table 1. Comparison of Phenol Antioxidant Quality (IC_{50}) and Lipoprotein-Bound Antioxidant Effectiveness (CLT_{50}) of Vitamins, Polyphenols, and Beverages[a]

compound or beverage	phenol content $\times 10^{-3}$(μM)	IC_{50} (μM)	CLT_{50} (μM)
ascorbic acid		1.45[a]	inactive
tocopherol		2.40[a]	54[b]
retinol		1.90	103
β carotene		4.30*a*	134
ubiquinol-10		1.33	65
epicatechin (tea)		0.19[a]	72[b]
catechin			85
epigallocatechin gallate (tea)		0.08[a]	42[b]
taxifolin (fruit juices)		0.34	38
quercetin (fruit juices)		0.22[a]	59[b]
quercetin rutinoside (citrus juice)		0.51[a]	108[b]
hesperetin (citrus juice)		3.66	inactive
hesperetin rutinoside (citrus juice)			inactive
cyanidin (red wine, grape juice)		0.21[a]	120[b]
resveratrol (red wine)		0.33[a]	59[b]
chlorogenic acid (coffee)		0.30[a]	108[b]
BEVERAGE			
orange juice	0.77	0.42	inactive
tangerine juice	1.38	0.50	inactive
grapefruit juice	1.59	0.95	inactive
prune juice	5.63	0.30	111
coffee	10.32	0.26	106
black tea	17.40	0.23	67
green tea	12.00	0.38	66
white grape juice	2.88	0.70	93
red grape juice	7.66	0.65	30
Bordeaux red wine	9.58	0.45	27
lager beer	10.13	0.30	390

[a] Vinson et al., 1995a. [b] Vinson et al., 1995b.

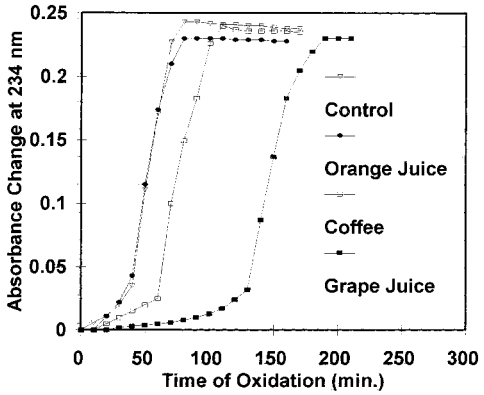

Figure 1. Ex vivo spiking of diluted beverages in plasma at 100 μM and the effect on the cupric ion oxidation of isolated LDL + VLDL as measured by conjugated diene formation at 234 nm.

VLDL as found previously for LDL (Romanchik et al., 1997). Although it is very lipophilic and binds to LDL, it was not very effective in decreasing the oxidative susceptibility of LDL isolated after ex vivo spiking.

The order of polyphenol effectiveness of lipoprotein-bound antioxidant activity is epigallocatechin gallate $\approx$ taxifolin > quercetin $\approx$ resveratrol $\gg$ quercetin rutinoside $\approx$ chlorogenic acid > cyanidin. Hesperetin and hesperetin rutinoside (hesperidin) did not exhibit any lipoprotein-bound antioxidant activity.

Representative lipoprotein oxidation curves after beverage spiking into plasma are shown in Figure 1. Among the beverages, the citrus juices such as orange, tangerine, and grapefruit did not have any antioxidant effect for the lipoproteins isolated after plasma spiking. This is not surprising since some major polyphenols in citrus juices are methoxyflavanones, some of which have been found to have no antioxidant activity (Benavente-García et al., 1997). The methoxyflavanone hesperetin did not have any lipoprotein-bound antioxidant activity. The best beverages were by far the red wine and red grape juice. These were followed by the two teas (green and black were equal) with beer being the poorest antioxidant in this model. The beverages, however, were quite good compared with the individual polyphenols present in the beverages. For instance, red wine was much better than cyanidin, which is an anthocyanin. Anthocyanins are the major class of antioxidant polyphenols present in red wine (Ghiselli et al., 1998). Thus, there may additive or synergistic effects of the polyphenols in the beverages when they bind to the lipoproteins which will be tested in future experiments.

The lipoprotein-bound antioxidant activity of the teas and wines shows that ex vivo plasma spiking can mimic the in vivo situation since drinking red wine (Fuhrman et al., 1995) and teas (Ishikawa et al., 1997) has been shown to produce in vivo LDL enrichment and a decreased LDL oxidizability. Conversely, orange juice, which had no lipoprotein-bound antioxidant activity in our model, had no in vivo lipoprotein antioxidant activity after human supplementation (Abbey et al., 1995). This ex vivo model is of course limited as the polyphenols in beverges are metabolized after absorption in vivo and therefore chemically changed. It remains to be shown whether the other active beverages in our model such as grape juice, prune juice, coffee, and beer have in vivo antioxidant activity.

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