ARTICLES

Plant Flavonoids, Especially Tea Flavonols, Are Powerful Antioxidants Using an *in Vitro* Oxidation Model for Heart Disease

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Flavonoids, commonly occurring antioxidants in foods, have been compared in a dose–response manner with vitamins C and E and β-carotene and found to be powerful antioxidants using an *in vitro* lipoprotein oxidation model. This model simulates the oxidation of low-density lipoproteins, which results in atherosclerosis. Of the flavonoids and flavonoid-related compounds, flavonols found in tea are the most powerful natural antioxidants. These results provide a mechanism for the beneficial epidemiological effect of dietary flavonoids on heart disease.

Keywords: *Flavonoid antioxidants*

INTRODUCTION

Plants synthesize the well-known antioxidants tocopherols, ascorbic acid, and carotenoids. Flavonoids are phenol derivatives synthesized in substantial amounts (0.5–1.5%) and widely distributed in plants. These compounds have been found to possess antioxidant and free radical scavenging activity in foods (Shahidi and Wanasundara, 1992). More than 4000 individual flavonoids have been identified in higher and lower plants (Harborne, 1988). They are hydroxylated, methoxylated, and/or glycosylated derivatives of the 2-phenylbenzo[*a*]pyrane ring, which consists of two benzene rings combined by mediation of the oxygen-containing pyrane ring. In general, the leaves, flowers, fruit, and other tissues of the plant contain glycosides, woody tissues contain aglycons, and seeds may contain either. They can be subdivided into six classes of flavonoids and flavonoid-related compounds: flavones, flavanones, isoflavones, flavonols, flavanols, and anthocyanins. The basic ring skeletons of these classes are shown in Figure 1. Flavonoids can be considered derivatives of flavonone with the B ring substituted on the 2-position of the heterocyclic ring except for isoflavonoids such as isoflavanone, in which it is substituted on the 3-position. Phenolic acids, while not possessing the ring structure of flavonoids, are phenolic antioxidants in foods and are often esterified with flavonoids.

Lower density lipoproteins, in the form of low-density lipoproteins (LDL) and very low density lipoproteins (VLDL), are hypothesized to be involved in the pathogenesis of atherosclerosis after oxidation (Steinberg *et al.*, 1989). There is substantial epidemiological as well as experimental evidence that increasing amounts of antioxidants in the diet such as vitamins C and E and β-carotene are preventive for heart disease (Manson *et al.*, 1993). Recently in a Dutch epidemiological study, dietary flavonoid intake, the majority from tea, was found to significantly reduce deaths from heart disease (Hertog *et al.*, 1993). Several flavonoids have been found to inhibit LDL oxidation *in vitro* by cupric ion and by macrophages (DeWhalley *et al.*, 1990; Mangiapane *et al.*, 1992). This research was undertaken to systematically compare flavonoids and other natural antioxidants in a lipoprotein oxidation model that simulates the oxidation mechanism of atherogenesis.

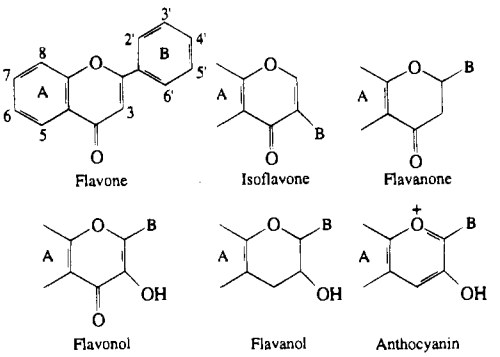

Figure 1. Flavonoid subclasses.

METHODS

Pure flavonoids and other antioxidants were obtained from either Sigma Chemical Co. or Aldrich Chemical Co. The tea compounds were a gift from Thomas J. Lipton Tea Co. Cyanidin chloride was synthesized and purified (King and White, 1957). Welch's Grape Color Extract, Type 250, was a gift of Welch's Co., and silymarin was donated by Madaus AG.

Lipoproteins LDL plus VLDL were isolated from the plasma of a single normocholesterolemic individual, who was not consuming antioxidant supplements, by using an affinity column (Isolabs, Inc.). The procedure for incubation of the oxidant cupric ion with the lipoproteins, analysis of the lipid peroxidation products, and determination of the concentration of antioxidant for 50% inhibition (IC_{50}) has previously been described (Vinson and Hontz, 1995). Phenol concentration in mixtures was determined with the Folin–Ciocalteu reagent (Sigma) using catechin as the standard.

RESULTS AND DISCUSSION

All compounds tested had a sigmoidal dose–response inhibition of lipoprotein oxidation. The IC_{50} data are

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Table 1. Comparison of Antioxidant Effectiveness of Vitamins, Flavonoids, and Phenols[a]

compound	OH substitution	IC_{50} (μM)
Vitamins and Synthetic Phenols		
butylated hydroxyanisole	mono	0.181
butylated hydroxytoluene	mono	0.270
trolox	mono	1.26
ascorbic acid (vitamin C)	vicinal	1.45
α-tocophenol (vitamin E)	mono	2.40
β-carotene (provitamin A)	none	4.30
Flavones		
genistein (isoflavone)	5,7,4′	14.3
apigenin	5,7,4′	>16
baicalein	5,6,7′	>16
chrysin	5,7	>16
flavone	none	>16
Flavanones		
hesperitin	5,7,3′	3.66
hesperidin (hesperetin rutinoside)	5,3′	>16
neohesperidin	5,3′	>16
naringenin	5,7,4′	>16
Flavonols		
quercetin	3,5,7,3′,4′	0.224
taxifolin (dihydroquercetin)	3,5,7,3′,4′	0.344
myricetin	3,5,7,3′,4′,5′	0.477
rutin (quercetin rutinoside)	5,7,3′,4′	0.512
morin	3,5,7,2′,4′	0.734
kaempferol	3,5,7,4′	1.82
Flavanols		
epigallocatechin 3-gallate	5,7,3′,4′,5′,3″,4″,5″	0.075
epigallocatechin	3,5,7,3′,4′,5′	0.097
epicatechin 3-gallate	5,7,3′,4′,3″,4″,5″	0.142
catechin	3,5,7,3′,4′	0.187
Anthocyanins		
cyanindin chloride	3,5,7,3′,4′	0.212
grape skin extract		0.951[b]
Phenolic Acids		
tannic acid (2–5 gallic esters with glucose)		0.152
caffeic acid (cinnamic acid)	3,4	0.241
nordihydroguairetic acid (pyrocatechol)	3,4,3′,4′	0.255
chlorogenic acid (cinnamic ester)	3,4	0.296
3,4-dihydrobenzoic acid	3,4	0.455
gallic acid (benzoic acid)	3,4,5	1.25
ellagic acid (gallic lactone)	one ortho, one mono	2.50
p-coumaric acid (cinnamic acid)	4	>16
Miscellaneous Phenols		
resveratrol (stilbene)	3,5,4′	0.332
gossypol (dinaphthalene)	two ortho, two mono	0.572
silymarin (flavolignan)	3,5,7, one mono	0.853
phloretin (propiophenone)	2,4,6,4′	1.08

[a] Antioxidants were added in duplicate at various concentrations to 70 μg/mL of lipoproteins in a pH 7.4 buffer with 25 μM cupric ion for a period of 6 h at 37 °C. Lipid peroxidation was measured by fluorescence of thiobarbituric acid reaction vs a control with no antioxidant added. The concentration for 50% inhibition was determined graphically. [b] Based on phenol concentration determined according to the Folin method.

shown in Table 1 for flavonoids and other natural and synthetic antioxidants. The listing within each group is in the order of decreasing antioxidant potency. In general, the well-known antioxidant vitamins were very poor antioxidants when compared to those flavonoids that had antioxidant activity. Trolox, a water-soluble form of vitamin E, was 2 times more potent than vitamin E, indicating some water solubility is advantageous in this oxidation model. The food additives BHT and BHA were very good antioxidants, probably because they are highly lipophilic and strongly bind to the lipoproteins.

Of the classes of flavonoids, the flavones from petals, leaves, and seeds had the poorest antioxidant activity due to the lack of o-dihydroxy groups. Genistein, an isoflavone from soybeans, was the only compound to show any activity. The flavanones, primarily found in citrus fruit, were the next poorest class, with only hesperitin possessing any significant antioxidant character.

The flavonols, commonly found flavonoids from flowers, leaves, and fruits, were good antioxidants. In addition to o-dihydroxy groups, they also have an o-hydroxyketo group that can chelate cupric ion (Thompson *et al.*, 1976), rendering it less oxidative. However, chelation is probably not the mechanism of action of flavonoids in our model since the effective antioxidant concentrations are 1–3 orders of magnitude lower than that of the cupric ion in the medium (25 μM). Rutin, the glycoside of quercetin, was a poorer antioxidant than quercetin. This was also found by Afanas'ev *et al.* (1989).

Phenolic acids (hydroxycinnamic, hydroxybenzoic, and ellagitannins) are found in many fruits and vegetables.

Chlorogenic and caffeic acids are present at 250 mg/cup of coffee (Ohnishi *et al.*, 1994). These compounds, with the exception of *p*-coumaric acid, which does not possess an *o*-dihydroxy group, were very good antioxidants. Phenolic acids have been shown by Laranjinha *et al.* (1994) to be consumed before LDL is oxidized, analogous to vitamin E.

Anthocyanins are responsible for the colors of flowers and fruits and are used as colorants for foods and pharmaceuticals as reviewed by Francis (1992). Although only one pure anthocyanin was tested, cyanidin proved to be a very good antioxidant. The grape skin extract was also a good antioxidant. Acylated malvidin glucoside, a major component of grape skins, was found to have twice the antioxidant power of tocopherol in a linoleic oxidation model (Tamura and Yamagami, 1994).

There were also three plant phenols studied that were not flavonoids. Gossypol from cottonseed and silymarin from milk thistle were good antioxidants. Resveratrol was a very good antioxidant and has been touted as an important antioxidant in red wines. Along with flavonoids, it has been hypothesized to be at least partially responsible for the health benefits of red wine (Frankel *et al.*, 1993a).

The flavanols, epicatechin polyphenols from tea, were the group of compounds containing the most powerful antioxidants. The activity increased with an increase in the number of *o*-dihydroxy groups. Epigalloepicatechin gallate, the most potent of all antioxidants tested, has four of these groups. It was 20 times more potent than the best vitamin, ascorbic acid.

The present work is the first to systematically compare flavonoids and flavonoid-related compounds to other phenols in a model that simulates the oxidation process leading to atherosclerosis and heart disease. Flavonoids are potentially beneficial with respect to the prevention of atherosclerosis, and these results provide a mechanism for the epidemiology study showing the benefits of flavonoids for heart disease. Studies on the antioxidant activity of flavonoids following supplementation are now in progress.

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