Antioxidative Effects of Plant Polyphenols

From Protection of G Protein Signaling to Prevention of Age-Related Pathologies

VIKTOR JEFREMOV, MIHKEL ZILMER, KERSTI ZILMER, NENAD BOGDANOVIC, AND ELLO KARELSON

aDepartment of Biochemistry, Tartu University, 50411 Tartu, Estonia
bDepartment of Neurology and Neurosurgery, Tartu University Hospital, 50411 Tartu, Estonia
cGeriatric Department, Neurotec, Karolinska Institute, Karolinska University Hospital, S-14186 Huddinge, Stockholm, Sweden

ABSTRACT: The antioxidant potency of three natural polyphenols, resveratrol, curcumin, and genistein, was compared by using the two human models: oxymodified with H2O2 and homocysteine (Hcy) G proteins in the postmortem frontal cortex (FC) membranes of age-matched control and Alzheimer’s disease (AD) subjects; and Cu2+-induced oxidation of plasma low-density lipoproteins (LDL). In Co, 3–10 μM polyphenols dose-dependently depressed the G protein 25% stimulation induced by 10 μM H2O2 or 500 μM Hcy. Resveratrol revealed significantly higher antioxidativity than curcumin or genistein. In AD, the antioxidativity of polyphenols showed no significant differences. Polyphenols (1 μM) significantly increased the LDL oxidation lag time (oxyresistance) as compared with control, the effect of resveratrol being most potent. Due to the dual antioxidant mechanism, the investigated polyphenols, particularly resveratrol, should have preferences for the preventive-therapeutic use in age-related oxidative stress-based pathologies.

KEYWORDS: polyphenols; antioxidant potency; human brain G proteins; human plasma LDL

INTRODUCTION

Aging is accepted as a nonmodifiable risk factor for the neurodegenerative (Alzheimer’s and Parkinson’s) and atherosclerosis-based cardiovascular...
The oxidative stress hypothesis of aging and age-related diseases suggests beneficial effects of antioxidative plant polyphenols in preventing and suppressing the accelerated aging, neurodegeneration, and atherogenesis. Recent data suggest that some dietary polyphenols, including phytoestrogens, exert dual effect: they improve the cognitive function in aging and in Alzheimer’s disease (AD) and reduce the atherogenic cardiovascular lesions. Many of the mechanisms behind these protective actions of the polyphenols have been elucidated in cellular and animal model systems, while only few mechanisms were shown in humans.

This study was undertaken to compare the antioxidant potency of three natural polyphenols, resveratrol (3,4′,5-trihydroxy-trans-stilbene), curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), and genistein (4′, 5, 7-trihydroxyisoflavone) (Fig. 1), by using the two human models: oxymodified with H$_2$O$_2$ and homocysteine (Hcy) G proteins in the postmortem brain membranes of age-matched control and AD subjects; and Cu$^{2+}$-induced oxidation of blood plasma low-density lipoproteins (LDL).

**MATERIALS AND METHODS**

The chemical reagents used were of highest analytical grade, mainly purchased from Sigma Chemical Co. (St. Louis, MO). $[^{35}\text{S}]-\text{GTP\gammaS}$ (1250 Ci/mmol) was obtained from NEN Life Science Products (Boston, MA).

The frontocortical (FC) tissues of postmortal human brain were obtained from Huddinge Brain Bank (Karolinska Institute, Stockholm, Sweden). The study included the tissues from 8 female control and 6 female AD patients (mean age ± SD, 82 ± 8, and 81 ± 7 years, respectively). The control group comprised subjects without history of psychiatric or neurologic disorders.

Preparation of the FC membranes and assay of $[^{35}\text{S}]-\text{GTP\gammaS}$ binding to the membrane G proteins were performed according to the described methods.
Briefly, the weighed FC tissue (100–120 mg) was homogenized in 6 volumes of 5 mM Tris-EDTA (TE) buffer (10 mM Tris-HCl and 0.1 mM EDTA, pH 7.4). The homogenates were diluted 1:10 with the same buffer, stirred on ice for 30 min, and centrifuged for 6 min at 1600 g. The membrane pellet was resuspended in TE buffer to give a protein concentration about 0.8–1.2 mg/mL, as determined by the method of Lowry. The $[^{35}\text{S}]-\text{GTP}\gamma\text{S}$ binding was estimated by incubation of FC membranes (final protein concentration 0.04 mg/mL) in a reaction cocktail containing TE buffer, 1 $\mu$M GDP, 1 mM dithiothreitol, 5 mM MgCl$_2$, 150 mM NaCl, and $[^{35}\text{S}]-\text{GTP}\gamma\text{S}$ (50,000–70,000 cpm in an aliquot of the reaction cocktail). Incubation was carried out for 10 min at 26°C in a total volume of 0.1 mL either in the absence (basal value) or in the presence of various concentrations of the polyphenols. Nonspecific binding was measured in the presence of 10 $\mu$M GTP$\gamma$S. Bound and free $[^{35}\text{S}]-\text{GTP}\gamma\text{S}$ were separated by vacuum filtration through GF/B filters (Whatman International Ltd., Mainstone, UK), which were washed three times with 5 mL of ice-cold TE-buffer. Radioactivity was quantified by “1414 WinSpectral” scintillation counter (FC & G Wallac, Turku, Finland). In data analysis, the basal $[^{35}\text{S}]-\text{GTP}\gamma\text{S}$ binding was defined as 100%.

To elucidate the effect of polyphenols on Hcy and H$_2$O$_2$ stimulation of $[^{35}\text{S}]-\text{GTP}\gamma\text{S}$ binding to control and AD FC membranes, the pro-oxidant-treated membranes were incubated with 3–10 $\mu$M polyphenols. The antioxidant effects were estimated as a difference in the stimulation of binding in the absence or presence of polyphenol. The effect of 1–10 $\mu$M polyphenols on basal $[^{35}\text{S}]-\text{GTP}\gamma\text{S}$ binding was studied in parallel.

LDL was isolated from the EDTA-separated fresh human plasma. One milliliter of plasma (diluted 1:1 with water) was mixed for 1 min with 0.1 mL protein precipitation reagent (2% dextran sulfate/2M MgCl$_2$ 1 : 1, pH 7.0), followed by mixing for 1 min and centrifugation at 1500 g for 10 min. In order to remove EDTA, the pellet was resuspended in 1 mL 0.9% phosphate-buffered saline (PBS) and reprecipitated by adding 0.05 mL precipitation reagent, vortexing, and centrifugation. The resulting LDL pellet was dissolved in 4% PBS. After the content of protein was assayed and adjusted to 2 mg/mL, the LDL fraction was immediately used for the study of oxidation kinetics. The kinetics of LDL oxidation was analyzed by adding 10 $\mu$M CuSO$_4$ to 200 $\mu$g/mL LDL protein. Formation of conjugated dienes (CD) was monitored continuously at 234 nm for up to 6 h at 37°C using a Jenway 6405 spectrophotometer (Barloworld Scientific Ltd., Essex, England). LDL oxidation kinetics in the presence and absence (control) of the plant polyphenols were analyzed on the basis of the oxidation lag time (interval between the initiation of oxidation and the intercept of tangent for the slope of the absorbance curve during the propagation phase). In data analysis, the lag time for the control LDL oxidation was defined as 100%.

The experimental data of the study were expressed as the mean ± standard error of the mean (SEM) for each statistical group (3–4 experiments in
duplicate). Student’s $t$-test for independent samples was used to determine significant differences between the groups. Values of $P < 0.05$ were taken to indicate significant difference.

**RESULTS**

In the FC membranes of control and AD brain, 1–10 μM of resveratrol, curcumin, and genistein induced a dose-dependent increase in $[^{35}S] \text{GTP} \gamma \text{S}$ binding, that is, in activity of G proteins (Fig. 2). In the control region, the 10 μM polyphenols stimulated G proteins with the maximal effects of 26, 24, and 14%, respectively. In AD region, the maximal stimulation of G proteins by curcumin revealed a significantly lower value than in control whereas the stimulation by two other agents revealed no significant decline. In control, each of the investigated polyphenols dose-dependently depressed the 25% stimulation of G proteins induced by 500 μM Hcy (Fig. 3) or 10 μM H$_2$O$_2$ (Fig. 4). At 10 μM concentration, resveratrol revealed the strongest antioxidant effect by depressing the Hcy- and H$_2$O$_2$-induced stimulation by 20 and 19%, respectively. Ten micromolar curcumin reduced the Hcy- and H$_2$O$_2$-induced stimulation of G proteins by 14% and 12% and 10 μM genistein by 9% and 10%, respectively. In AD, antioxidant effect of resveratrol on the Hcy and H$_2$O$_2$ stimulation of G proteins was significantly ($P < 0.05$) lower (10% and 11%, respectively) than in control whereas the effect of other two polyphenols showed insignificant decline (Figs. 3 and 4). Thus, the differences in the polyphenols antioxidant potency toward G proteins are tended to get lost in AD brain membranes.

In the plasma concentration (0.5 and 1 μM), resveratrol, curcumin, and genistein significantly increased the resistance of LDL to Cu$^{2+}$-induced

**FIGURE 2.** Stimulatory effect of 1–10 μM resveratrol, curcumin, and genistein on the $[^{35}S] \text{GTP} \gamma \text{S}$ binding in female control (Co) and AD FC membranes (100% = basal GTP$[^{35}S] \gamma \text{S}$ binding); $n = 4–5$. 

![Graph A](image1.png)  
(A)  

![Graph B](image2.png)  
(B)
FIGURE 3. Effect of 10 μM resveratrol, curcumin, and genistein on GTP$^{[35S]}$ binding in control and AD FC membranes treated with 500 μM Hcy at 26°C for 5 min. 100% = basal $^{[35S]}$GTPγS binding; n = 3–4. *P < 0.05 versus Hcy-treated value.

oxidation. The effect was manifested by a prolongation of the oxidation lag time showing the suppression of radical chain reactions in LDL lipids. Figure 5 shows, that treatment with 1 μM resveratrol produced the greatest 4.8-fold prolongation in LDL oxidation lag time as compared with control LDL. A 1 μM curcumin and genistein caused the 2.8- and 1.5-fold prolongation, respectively. Thus, the investigated polyphenols reveal different (structure-dependent) antioxidativity (antiatherogenicity) toward LDL oxidation, the resveratrol being the most potent in this respect.

DISCUSSION

The oxidative stress hypothesis of aging and age-related diseases suggests beneficial effects of antioxidative plant polyphenols in preventing and suppressing the neurodegeneration and atherogenesis.3–5 These effects of polyphenols have been mainly demonstrated in cellular and animal models of the pathologies whereas only few investigations were made with human subjects.8,9 This study provides antioxidant mechanisms for three plant polyphenols (resveratrol, curcumin, and genistein) by using two human models: oxy-modification of G proteins in the membranes of human brain FC and oxidation of human plasma LDL.

Our results showed that all three plant polyphenols dose-dependently increase the activity of G proteins in FC membranes. The stimulatory effect was found to be stronger in normal aging than in AD. The fact that AD progression leads to the remarkable dysregulation of G proteins in human FC
and other brain regions prone to injury, might explain the lowered G protein response to the polyphenols in AD (as compared to control). In both, control and AD brain, the stimulatory potency of three polyphenols revealed structure-dependent variations with the effect of stilbene resveratrol (FIG. 1) being more potent than the effect of curcumin (diarylheptane) or genistein (isoflavone). Many plant polyphenols (including resveratrol, curcumin, and genistein) have been shown to behave as agonists and/or modulators of estrogen receptors in the brain and periphery. In addition, the brain membrane estrogen receptors appear to be coupled to G proteins. These facts and the consideration that most of the nutritional polyphenols are able to cross the blood–brain barrier allow to suggest that the neuroprotective (and neurotrophic) effects of polyphenols involve the stimulation of the estrogen receptor G protein–mediated signaling cascades as a mechanism.

Previous studies have shown that severe oxidative modification of G proteins in the model systems of neurodegeneration can initiate/promote the neuronal death signals. Our study revealed that oxidative stimulation of G proteins in the brain membranes by metabolic pro-oxidants, Hcy and H$_2$O$_2$, probably occurring in accelerated aging and in neurodegeneration, can be significantly depressed by resveratrol, curcumin, and genistein. In control, the polyphenols revealed remarkable structure-dependent differences in their protective (antioxidant) effect against the G proteins oxymodification. Resveratrol revealed the higher antioxidant effect toward the oxymodification than curcumin whereas the effect of genistein tended to be the lowest. The partial explanation for such a structure–activity relationship comes from the fact that isoflavone genistein does not possess ortho- or paradiphenolic moieties which are important for the polyphenol antioxidant activity. However, in AD FC, the

![FIGURE 4. Effect of 10 μM resveratrol, curcumin, and genistein on GTP[$^{35}$S]γS binding in control and AD FC membranes treated with 10 μM hydrogen peroxide (H$_2$O$_2$) at 30°C for 5 min. 100% = basal [${}^{35}$S]GTPγS binding; n = 3–4. *P < 0.05 versus H$_2$O$_2$-treated value.](image)
FIGURE 5. Effects of 0.5 μM and 1 μM resveratrol, curcumin, and genistein on the LDL oxidation lag time. LDL fraction of the human plasma was oxidized by adding 10 μM CuSO₄ to 200 μg/mL LDL protein (100% = lag time for the control LDL oxidation); n = 4. Significant differences compared to the control are indicated by *P < 0.05, **P < 0.01, and ***P < 0.005.

antioxidant potency of the investigated polyphenols showed no significant differences. Probably due to the reduced level of oxidable substrates in the more injured AD brain regions (as compared with age-matched control), the oxidizing (stimulatory) effect of metabolic pro-oxidants on G proteins in AD was lower than in control (FIGS. 3 and 4) and, thereby, the structure-caused differences in antioxidant behavior of the three polyphenols could not be manifested. Yet, we suggest that our findings have an important implication for choosing the plant polyphenols (and their synthetic analogs) as preventive-therapeutic agents against the accelerated brain aging and neurodegeneration.

Of particular importance is our finding that the plasma concentrations of three polyphenols significantly increased the oxyresistance of LDL. This is consistent with previous studies demonstrating the significant antioxidant behavior of some phytoestrogens toward LDL oxidative susceptibility.²⁰,²¹ Interestingly, resveratrol appeared to be the most potent antioxidant in respect to LDL oxidation (compared with other polyphenols), the feature being similar to that found in depressing the oxymodification of brain G proteins by
the polyphenols. It is widely accepted that oxidative modification of LDL contributes to the atherogenesis and the atherosclerosis-based diseases. We consider that inhibition of LDL oxymodification by regular supplementation of antioxidative dietary polyphenols represents a potential preventive-therapeutic possibility against the progression of atherosclerotic lesions in the cardiovascular system.

Taken together, the three plant polyphenols studied have a structure-dependent antioxidant potency that might protect the human brain G protein–mediated signaling as well as the human plasma LDL from the deleterious oxidative modifications. Due to the dual antioxidant mechanism, resveratrol, curcumin, and genistein should have preferences for the preventive-therapeutic use in age-related oxidative stress-based pathologies (neurodegeneration, atherogenesis, etc.). Among the other polyphenols, resveratrol appears to be most effective in limiting the development of these pathologies.

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