Cardioprotective effect of natural antioxidants: evaluation in cultured cardiomyocytes

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Introduction

The average diet contains a great number of compounds with antioxidant activity[1], among which polyphenols, plant metabolites occurring widely in plant food, possess outstanding antioxidant and free radical scavenging properties suggesting a possible protective role in man[2]. Green tea is an excellent source of polyphenols, particularly of a group of compounds known as green tea catechins (GTCs)[3]. The content of the different GTC isomers could vary among different green teas, depending on the species, the climate, the cultural practices and, in the case of green tea extracts (GTEs), also on the condition and the technology used for the extraction and the conservation. Therefore, the antioxidant ability of different GTEs could vary according to their GTC quali-quantitative composition.

The purpose of this study was to evaluate the ability of different GTEs in reducing free radical-mediated damage in comparison to a well known antioxidant agent, α-tocopherol[4], using cultured cardiomyocytes as an experimental model system. Cultured cardiomyocytes are a suitable model to evaluate the possibility of counteracting peroxidative damage by the supplementation with exogenous antioxidants since free radical-induced damage is implicated in different cardiovascular diseases (i.e. ischemia/reperfusion, cardiomyopathy, etc.), and the heart has low antioxidant defenses[5]. Primary cultures of neonatal rat cardiomyocytes were exposed to a free radical generating system (FRGS) catalyzed by xanthine oxidase, in the presence of different GTEs and α-tocopherol. The protective effect of the different supplementations was evaluated by measuring the conjugated diene production, as an index of lipid peroxidation, and the lactate dehydrogenase (LDH) release from cell cultures. Furthermore, GTEs were analyzed by reverse phase high performance liquid chromatography (HPLC), and their qualitative and quantitative composition was correlated with their antioxidant ability in cardiomyocytes.

Methods

Primary heart cell cultures were obtained by the isolation of cardiomyocytes from the ventricles of 2-4-day-old Wistar rats and grown as previously reported[6]. At confluence, cardiomyocytes were supplemented with 20 μM α-tocopherol or 10 or 50 μg/ml of the different aqueous solutions of GTEs. Twenty-four hours later, cultures were exposed for 1 hour to the FRGS (2.3 mM purine, 2.4 μM Fe^{3+} loaded transferrin and 0.01 U/ml xanthine oxidase). Appropriate control groups were likewise processed in the absence of α-tocopherol and GTEs and/or FRGS. All the cells were scraped off in ice-cold methanol, and the appearance of conjugated diene-containing lipids and LDH release were evaluated as reported by Bordoni et al.[7]. Data are expressed as means – SD of five different cultures and statistical differences were evaluated using the Student s t test.

The HPLC analyses of GTEs were performed on a Jasco model LC 1600 (Tokyo, Japan), equipped with a Luna™ 5 μm C18

column (Phenomenex, Torrance, CA, USA) and a Jasco diode-array UV-VIS detector model MD1510 at 270 nm. The GTEs were dissolved in distilled water/formic acid 99.7/0.3 v/v (1 mg/ml), and sonicated for 10 min at 30 C at 50-60 Hz. A linear gradient elution was carried out using double distilled water/methanol/formic acid (74.7/25/0.3 v/v) and acetonitrile/formic acid (99.7/0.3 v/v). Identification of GTCs was performed by comparison of the retention times of the unknown peaks to reference standards. Data are expressed as means – SE of four analyses.

Results

Conjugated diene levels are reported in figure 1 as the fold increase in conjugated diene production in non-supplemented and antioxidant supplemented cell cultures exposed to the FRGS, in comparison to their respective counterparts not exposed to the FRGS. No differences in diene content were found between different groups when cardiomyocytes were not exposed to the oxidative stress (A_{235nm} 0.067 – 0.003). Exposure to the FRGS caused a 4-fold increase in conjugated diene production in cardiomyocytes not supplemented with antioxidants. In the presence of α-tocopherol, a significant attenuation of conjugated diene formation was detected; supplementation with the different GTEs revealed the ability of these natural antioxidants to protect cardiomyocytes against peroxidative damage, although to a different extent. The protective effect of GTEs was dependent on the concentration used, and it was higher using GTE 3, than GTE 2 and 1. The supplementation with 50 μg/ml GTE 3 had a similar protective effect to α-tocopherol.

The release of LDH from non-supplemented and antioxidant supplemented cardiomyocytes exposed to the FRGS is reported in figure 2 as the fold increase in comparison to their counterparts not exposed to the oxidative stress. No differences in LDH release were detected in cultures not exposed to the FRGS (37.5 – 2.5 U/ml). A significant LDH release was demonstrated after exposure to the oxidative stress in the absence of the antioxidants, while, in cardiomyocytes supplemented with α-tocopherol, LDH release was similar to that of cells not exposed to the oxidative stress. A protection was also achieved by the addition of GTEs; in agreement with data on conjugated diene production, GTE 3 demonstrated the highest protective effect; when it was supplemented at 50 μg/ml, LDH release was similar to that obtained in the presence of α-tocopherol.

HPLC analysis revealed a total catechin content (mg/g GTE) of 93.0 – 3.73, 697.5 – 16.56 and 757.6 – 12.34 for GTE 1, 2 and 3 respectively. Figure 3 represents the composition in single catechins of the three GTEs. GTE 1, revealing the lowest total and single catechin content, is clearly a non-concentrated extract. (-)-epigallocatechin-3-gallate and (-)-epicatechingallate were the most representative catechins in GTE 2 and 3. Furthermore, GTE 3 was the only extract with high levels of (-)-gallocatechingallate, while GTE 2 revealed higher levels of (-)-epigallocatechin than GTE 1 and 3.

Discussion

Although the assimilation, metabolic fate and toxicity of antioxidants supplied by the human diet are not well defined yet, their constant consumption warrants the consideration of their physiological role, especially when

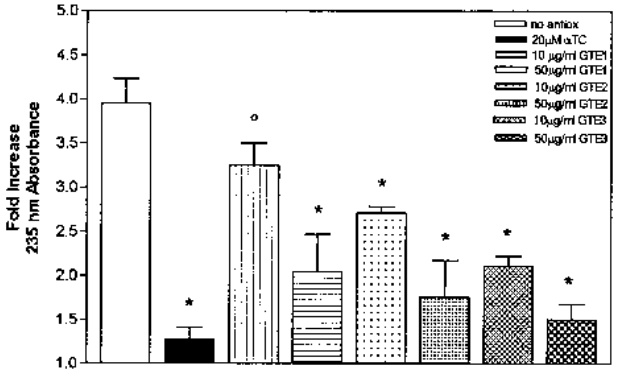

Figure 1. *Fold increase in conjugated diene content of neonatal rat cardiomyocytes exposed to the free radical generating system (FRGS) for 1 hour. Conjugated diene production was measured as 235 nm absorbance as reported in the Methods section. Data are expressed as means – SD of five different cell cultures. Statistical analysis was performed by the Student s t test comparing cardiomyocytes treated and non-treated with different antioxidants and exposed to FRGS ($p < 0.01$, * $p < 0.001$). In comparison to the corresponding non FRGS-exposed cells, statistical analysis revealed a significant increase in conjugated diene production: $p < 0.05$ in α-tocopherol (αTC) and 50 μg/ml green tea extract (GTE) 3 treated cells; $p < 0.01$ in 10 μg/ml GTE 3 and 50 μg/ml GTE 1 and 2 treated cells; $p < 0.001$ in non-treated and in 10 μg/ml GTE*

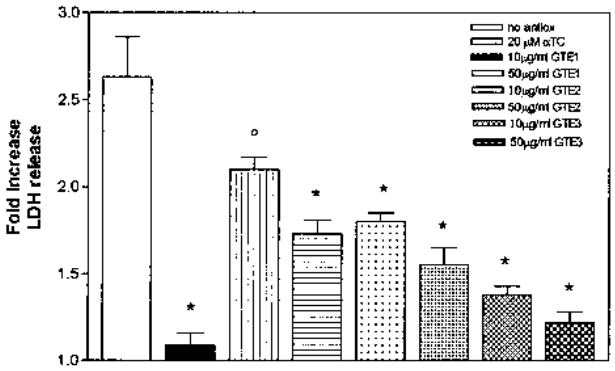

Figure 2. *Fold increase in lactate dehydrogenase (LDH) release in the culture medium of neonatal rat cardiomyocytes exposed to the FRGS for 1 hour. LDH release was measured on aliquots of culture media as reported in the Methods section. Data are expressed as means – SD of five different cell cultures. Statistical analysis was performed by the Student s t test comparing cardiomyocytes treated and non-treated with the different antioxidants and exposed to FRGS ($p < 0.01$, * $p < 0.001$). In comparison to the corresponding non-FRGS-exposed cells, statistical analysis revealed a significant increase in LDH release apart from cells treated with αTC: $p < 0.05$ in 50 μg/ml GTE 3; $p < 0.01$ in 10 μg/ml GTE 3 and 50 μg/ml GTE 2 treated cells; $p < 0.001$ in non-treated, 10 μg/ml GTE 1 and 2 and 50 μg/ml GTE 1. Abbreviations as in figure 1.*

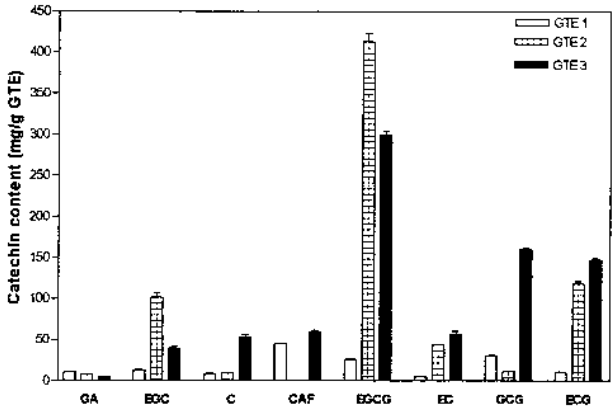

Figure 3. *Catechin composition of the different green tea extracts (GTEs). Catechin composition analysis was performed by reverse phase high performance liquid chromatography as reported in the Methods section. Values are reported as mg/g dry GTE and are means – SE of four analyses. C = catechin; CAF = caffeine; EC = epicatechin; ECG = epicatechingallate; EGC = epigallocatechin; EGCG = epigallocatechin-3-gallate; GA = gallic acid; GCG = gallocatechingallate.*

they are as effective as well-known antioxidants like α-tocopherol.

The exposure of cardiomyocytes not supplemented with antioxidants to FRGS, able to yield superoxide generation at levels comparable to *in vivo* production by stimulated neutrophils[8], and hydroxyl radical generation[9], caused a significant increase in conjugated diene production and in LDH release in the medium. The addition of α-tocopherol almost completely protected cardiomyocytes, and also all the GTEs tested exerted a protection against peroxidative damage, but to a different extent and in a dose-dependent manner: GTE 3 at a 50 μg/ml concentration had the maximum effect, comparable to α-tocopherol.

As evidenced by HPLC analysis, the GTEs showed not only a different total catechin content, but also significant differences in their qualitative-quantitative composition. The weak antioxidant activity of GTE 1 appears to be related to its low total catechin content; otherwise, since GTE 2 and 3 have similar total catechin content, their different antioxidant effectiveness may be related to differences in single catechin content. Many studies have related the antioxidant activity of the different catechins to their chemical structure[10,11]. According to these studies, the antioxidant activity of GTE 2 and 3 could be related to their high content of epicatechingallate and epigallocatechin-3-gallate; the higher antioxidant effectiveness of GTE 3 than GTE 2 could be tentatively related to its higher content in catechin, epicatechin and gallocatechingallate. Alternatively, since the antioxidant activity of GTEs is probably due to the synergistic effect of all the components, the higher activity of GTE 3 could not be related to the highest content of a single catechin but to a most advantageous overall distribution of all components.

Among primary concerns to be addressed before antioxidant nutritional supplementation or food fortification is recommended as a chronic disease prevention strategy for the general population, is the real protective effect of these antioxidants compared to well defined antioxidants such as α-tocopherol, and antioxidant organ specificity. Based on our results, GTE 3 presents a protective effect similar to α-tocopherol and can be considered a highly protective antioxidant at cardiac level. Therefore GTEs with quali-quantitative composition similar to GTE 3 could be of great importance in human nutrition and preventive medicine, particularly in the cardiovascular system, in the light of the very low antioxidant defenses of the heart.

References

1. Bonorden WR, Pariza MW. Antioxidant nutrients and protection from free radicals. In: Kotsonis FN, Mackey M, Hjelle J, eds. Nutritional toxicology. New York, NY: Raven Press, 1994: 19-48.
2. Scott BC, Butler J, Hallywell B, Aruoma OI. Evaluation of the antioxidant action of ferulic acid and catechin. Free Rad Res Commun 1993; 19: 241-53.
3. Zhu QY, Zhang A, Tsang D, Huang Y, Chen ZY. Stability of green tea catechins. J Agric Food Chem 1997; 45: 4624-8.
4. Massey KD, Burton KP. Free radical damage in neonatal rat cardiac myocyte culture: effects of α-tocopherol, trolox and phytol. Free Rad Biol Med 1990; 8: 449-58.
5. Dorr RT. Cytoprotective agents for anthracyclines. Semin Oncol 1996; 23: 23-34.
6. Bordoni A, Biagi PL, Rossi CA, Hrelia S. Alpha-1-stimulated phosphoinositide breakdown in cultured cardiomyocytes: diacylglycerol production and composition in docosahexaenoic acid supplemented cells. Biochem Biophys Res Commun 1991; 174: 869-77.
7. Bordoni A, Biagi PL, Hrelia S. The impairment of essential fatty acid metabolism as a key factor in doxorubicin-induced damage in cultured rat cardiomyocytes. Biochim Biophys Acta 1999; 1440: 100-6.
8. Tate RM, Yanbenthuysen KM, Shasby DM, McMurty IF, Repine JE. Oxygen-radical-mediated permeability edema and vasoconstriction in isolated perfused rabbit lungs. Am Rev Respir Dis 1982; 126: 802-6.
9. Burton KP, McCord JM, Ghay G. Myocardial alterations due to free radical generation. Am J Physiol 1984; 246: H776-H783.
10. Shahidi F, Wanasundara JP. Phenolic antioxidants. Crit Rev Food Sci Nutr 1992; 32: 67-103.
11. Salah N, Miller NJ, Paganga G, Tijburg L, Bolwell GP, Rice-Evans C. Polyphenolic flavonols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. Arch Biochem Biophys 1995; 322: 339-46.