Life Sciences 126 (2015) 1–9

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Concentrated green tea supplement: Biological activity and molecular mechanisms

S. Cyboran [a,*], P. Strugała [a], A. Włoch [a], J. Oszmiański [b], H. Kleszczyńska [a]

[a] Department of Physics and Biophysics, Wrocław University of Environmental and Life Sciences, Norwida 25, 50-375 Wrocław, Poland
[b] Department of Fruit, Vegetable and Cereal Technology, Wrocław University of Environmental and Life Sciences, Norwida 25, 50-375 Wrocław, Poland

ARTICLE INFO

Article history:
Received 14 July 2014
Accepted 17 December 2014
Available online 20 February 2015

Chemical compounds studied in this article:
(−) Epigallocatechin gallate (PubChem CID: 65064)
Gallic acid (PubChem CID: 370)

Keywords:
Green tea supplement
Antioxidant activity
Anti-inflammatory activity
UPLC
Polyphenols
Membrane fluidity
Erythrocytes
Hemolytic activity

ABSTRACT

Aim: This study was undertaken to determine the biological activity of a green tea supplement with respect to cells and erythrocyte membranes and the molecular mechanism of that activity.
Main methods: The extract's activity was evaluated on the basis of its hemolytic, antioxidant and anti-inflammatory actions. In addition, the extract's effect on the physical properties of the erythrocyte membrane was examined. We also conducted a detailed analysis of supplement ingredients using high-yield liquid chromatography, supplemented with standard tests of total content of polyphenols and flavonoids in the supplement.
Key findings: The study showed that green tea extract has a high antioxidant and anti-inflammatory capacity with no deleterious effect on red blood cells. The extract modifies the physical properties of the erythrocyte membrane, apparently by binding to its hydrophilic region, with consequent rigidity of the hydrophobic region, increased hydration and a moderate increase in its resistance to changes in tonicity of the medium. Because the extract's components anchor in the polar region of membrane lipids, they are able to effectively scavenge free radicals in the immediate vicinity of the membrane and hinder their diffusion into its interior.
Significance: Green tea supplement at concentrations markedly exceeding the blood plasma physiological polyphenol concentrations has no destructive effect on the erythrocyte membrane. Due to the high content of flavan-3-ols, the supplement exhibits high biological activity, which makes it an alternative source of those substances to the commonly used infusion of green tea leaves.

Introduction

Green tea is a rich source of flavan-3-ols, and its extracts are widely used in the prevention and treatment of many diseases. They have anti-inflammatory, antibacterial and antiviral effects, and prevent the development of cancer and diabetes [21,25,39,54,59,65,77].

In recent years, however, there have been reports that green tea polyphenols, when consumed in large quantities, cause unwanted side effects in the body [18,22,26,51]. Commonly available supplements and plant extracts are not subject to stringent requirements, and therefore they may be sold without detailed studies determining their effect on biological systems. Both bioprotective and negative effects of consumed plant supplements probably depend on a number of factors, not just the size of the dose intake. In addition, research has shown that the biological activity of phenolics contained in green tea is significantly different from the isolated single components [61,76,78]. Green tea ingredients such as epigallocatechin gallate (EGCG) and gallic acid (GA) have both protective and therapeutic properties, as well as being damaging to cells and their membranes [7,11,24,34,35,38,41]. Current knowledge does not, however, allow a full explanation of the healthy effects and negative ones induced by extracts of green tea.

Despite the large amount of information in the worldwide scientific articles on biological activity of extracts of green tea and polyphenolic compounds contained therein, the mechanism of molecular interaction of these substances in the body is not yet fully explained. In particular, there is no definite explanation of the effects of concentrated extracts of green tea. Information regarding the impact of such extracts on cell membrane properties is also lacking. Therefore it seems beneficial to determine the biological activity of concentrated green tea extract contained in a supplement with a focus on cells and cell membranes, in order to determine the mechanism of its action on biological systems.

Composition of phenolic compounds in green tea supplement was defined using different methods. Biological activity of the supplement was defined in biophysical studies, and to indicate which substances are mainly responsible for the activity, the research was conducted on two selected components of the supplement: (−) epigallocatechin-3-gallate (EGCG) and gallic acid (GA). These compounds were chosen because they represent two different groups of polyphenolic compounds found in green tea; EGCG belongs to flavan-3-ols and GA to phenolic acids. Biological activity of the selected substances was established on

* Corresponding author. Tel.: +48 713205275; fax: +48 713205167.
E-mail address: sylwia.cyboran@up.wroc.pl (S. Cyboran).

http://dx.doi.org/10.1016/j.lfs.2014.12.025
0024-3205/

the basis of hemolytic, antioxidant and anti-inflammatory activities and their impact on the physical properties of the erythrocyte membrane.

Materials and methods

Green tea supplement, polyphenols

A dietary supplement widely available on the world market, which contains green tea and high contents of polyphenolic compounds, was used in the study. One supplement capsule contains 300 mg of polyphenol extract of green tea. The raw material used to manufacture this extract comes from the leaves and stems of green tea growing in the mountainous areas of the province of *Fujian* (*South China*). The dominant species of green tea in the material from which the polyphenols were extracted is Tieguanyin. EGCG, (+) catechin, (−) epicatechin (EC), quercetin-3-rutinoside (rutin) and gallic acid (GA) were purchased from Extrasynthese, Paris Cedex, France.

Cells and biological membranes

The investigation was conducted on erythrocytes and their membranes obtained from fresh heparinized pig blood according to the method of Dodge et al. [15]. The content of erythrocyte membranes in the samples was determined on the basis of protein concentration, which was assayed using the Bradford method [8], and it was 100 μg/ml. The choice of pig erythrocytes was dictated by the fact that this cell's percentage content of lipids is closest to that of the human erythrocyte, and the blood readily available. Each time, fresh blood was added to a physiological solution of sodium chloride containing heparin.

Fluorescence probes, enzymes and reagents

The fluorescent probes Laurdan (6-dodecanoyl-2-dimethylaminonaphthalene), DPH (1,6-diphenyl-1,3,5-hexatriene), and TMA-DPH (1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene p-toluenesulfonate) were purchased from Molecular Probes, Eugene, Oregon, USA. The Folin–Ciocalteu phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-diazobis (2-amidinopropane) dihydrochloride (AAPH), butylated hydroxyanisole (BHA), L(+) ascorbic acid (AA), and enzymes COX-1, COX-2 and 1-LOX were purchased from Sigma-Aldrich, Inc., Steinheim, Germany. All other chemicals were of analytical grade, obtained from Sigma-Aldrich, Inc., Steinheim, Germany.

Phenolic content

Total phenolic content

Total phenolic content was determined using the Folin–Ciocalteu (F–C) reagent, adapted from Blainsky et al. [5]. The standard curve was made for gallic acid. The results were expressed as mg gallic acid equivalents (GAE) per 1 g of dry sample.

Total flavonoid content

GT extract was analyzed for total flavonoid content according to the colorimetric method described previously by Lamaison and Carnat [33].The standard curve was prepared for rutin (quercetin-3-0-rutinoside) under the same conditions. The results were expressed as mg rutin equivalents (Q3R) per 1 g of dry sample.

UPLC–DAD and UPLC–ESI-MS analyses

The percentage content of polyphenols in the extract of green tea was determined by means of UPLC/DAD and the UPLC/ESI/MS method analyses described by Oszmiański et al. [49].

Hemolytic activity

The ability of polyphenolic compounds contained in the GT extract, EGCG and GA to induce hemolysis of red blood cells was studied using spectrophotometric methods described earlier in Kleszczyńska et al. [30] with minor modifications.

Levels of hemolysis of blood cells modified with the substances at concentrations of 0.005 to 0.1 mg/ml and of the control were determined after different times of incubation. Briefly, the modification was conducted at 37 °C thrice (1 h, 2 h and 3 h), each sample containing 1 ml of erythrocyte suspension of 1.2% hematocrit, stirred continuously. After modification, 2 ml of isotonic phosphate buffer of pH = 7.4 was added to each sample. Next, the samples were centrifuged and the supernatant assayed for hemoglobin content using a spectrophotometer (Specord 40, Analytik Jena) at a 540 nm wavelength. Hemoglobin concentration in the supernatant, expressed as percentage of hemoglobin concentration in the supernatant of totally hemolyzed cells, was assumed as the measure of the extent of hemolysis.

Antioxidant activity

DPPH radical scavenging activity assay

The effect of studied substances on reduction of DPPH• radical concentration was measured spectrophotometrically, as previously described by Vidal et al. [71].

In that study, the measure of antioxidant activity of the studied substances was assumed to be EC_{50} — the concentration at which the free radicals DPPH• are reduced by 50%.

Inhibition of membrane lipid peroxidation

Antioxidant activities of GT extract, GA, and EGCG were determined using the fluorimetric method described in our previous work [6] with minor modifications. The studies were carried out on membranes of erythrocytes (RBC). The TMA-DPH probe was used in these experiments. Suspensions of erythrocyte membranes (ghosts) were treated with the chemical oxidation inductor AAPH for 30 min at 37 °C after addition of appropriate amounts of antioxidants (GT extract, GA, EGCG dissolved in ethanol). Free radicals generated by thermal decomposition of AAPH at 37 °C attacked the erythrocyte membranes and induced lipid peroxidation. They also caused quenching of TMA-DPH fluorescence and decreased fluorescence intensity. A Cary Eclipse (Varian) spectrofluorimeter was used to measure free radical concentrations in the samples. Excitation and emission wavelengths were $\lambda_{ex} = 362$ nm and $\lambda_{em} = 428$ nm. The measure of lipid oxidation was the relative change of fluorescence intensity, F/F_0, where F_0 is the initial fluorescence and F the one measured during an oxidation procedure [2]. The concentration of the compounds (IC_{50}) at which 50% inhibition of peroxidation occurred (fall in fluorescence intensity) was assumed as a measure of their antioxidant activity. The results of the assays were expressed relative to Trolox® and AA.

Inhibition of enzyme activity

Cyclooxygenase inhibitory activity

The anti-inflammatory activity of the GT supplement and EGCG, established on the basis of a modified method given in the work by Kumar et al. [31], was assayed with a spectrophotometric measurement of inhibition of activity of the cyclooxygenase COX-1 and COX-2. In short, the experiment was conducted as follows: into a cuvette containing Tris–HCl buffer (pH 8.0) the following were successively added: the studied enzyme inhibitor (GT, EGCG at 2.5 mg/ml initial concentration), hematin (0.1026 mM) and cyclooxygenase (COX-1 or COX-2) at 1 mg/ml. After mixing and incubation (approx. 3 min), TMPD was added at 24.35 mM. To initiate the inhibition reaction, arachidonic acid was added at a concentration of 35 mM. The final volume of the sample was 1 ml. Changes in absorbance of the sample were