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Anti-atherosclerotic activity of catechins depends on their stereoisomerism

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ABSTRACT

In terms of stereochemistry, catechins are divided into two groups: (−) epi forms (2R, 3R) and (−) forms (2S, 3R). Most of the catechins present in green tea are (−) epi forms (2R, 3R). Under the influence of high temperatures, in anaerobic conditions, as a result of epimerization the proportion of the (−) form (2S, 3R) increases. The data indicate that the presence of thermally modified catechins in the diet more efficiently reduces the development of atherosclerosis in apoE knockout mice than the presence of native catechins. The addition of the thermally modified formulations to the high-fat diet resulted in a reduction of the area of atherosclerotic lesions by about 28% (en face method) and 45% (cross-section method) compared to the group fed the high-fat diet without catechins. Furthermore, the body weight gain and plasma TBARS concentration in mice fed a diet with the addition of catechins depends on the degree of epimerization of catechins and decreases with increasing content of catechins belonging to the (−) form (2S, 3R). Moreover, plasma HDL cholesterol concentration in mice depends on catechins' stereoisomerism and increases along with the increasing content of catechins belonging to the (−) form (2S, 3R).

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1. Introduction

Catechins are the major group of polyphenols of tea leaves, constituting 20–30% of the dry weight of the leaf. In terms of stereochemistry, catechins are divided into two groups: (−) epi forms (2R, 3R) and (−) forms (2S, 3R). (−) Epi forms (2R, 3R) constitute about 90% of catechins naturally occurring in the tea leaf. Under the influence of high temperatures, in anaerobic conditions, as a result of epimerization the proportion of the (−) form (2S, 3R) increases [1]. The stereoisomerism of catechins affects their properties. It has been shown that the thermally modified catechin formulations used as food stabilizers more efficiently prevent oxidative and hydrolytic processes occurring in the fats during their storage than the native catechins formulations [2]. A greater antioxidative potential of the thermally modified catechins formulations than native catechins formulations is likely to be the result of a synergistic effect of the compounds of both groups of stereoisomers, and it is not caused by the increased activity of the individual catechins belonging to the (−) form (2S, 2R). This is indicated by the results obtained in studies comparing the antioxidant effectiveness of different pairs of stereoisomers. Xu et al. found that catechins within individual pairs of stereoisomers have comparable antioxidant potential; only the pairs EGC and GC showed a difference in favor of epi forms [3].

In the literature there are many reports indicating the health-related (anti-atherosclerotic, anti-cancer, anti-obesity and even anti-diabetic) properties of green tea catechins [4]. Anti-atherosclerotic effects of catechins, among others, are the result of the impact they have on the metabolism of cholesterol. Green tea catechins cause a decrease blood plasma total cholesterol level in experimental animals and in humans [5,6]. Moreover, it has been revealed that thermally modified catechins added to the diet of rats reduce total cholesterol [5] and triglycerides [7] in plasma more effectively than native catechins. Lowering the level of cholesterol is a complex process and is a result of the effects catechins on: the decreased absorption of exogenous and endogenous cholesterol from the digestive tract [8,9], the reduction of bile acid resorption in the intestine by the inhibitory effect on the apical sodium bile acid transporter (ASBT) [10], and inhibiting the activity of HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl-CoA reductase), which is

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the key enzyme responsible for the synthesis of cholesterol in the liver [11]. Reducing the absorption of exogenous and endogenous cholesterol from the digestive tract is the result of formation of cholesterol conjugates with catechins [8] and the inhibitory activity of catechins on phospholipase A2 [9], which allows the catalytic activity of cholesterol esterase and significantly enhances absorption of cholesterol by enterocytes.

Anti-atherosclerotic effects of green tea catechins have been confirmed in a study conducted on apoE knockout mice [12–15]. However, an interesting topic, so far unexplained, is the impact of catechins' stereoisomerism on their anti-atherosclerotic potency. The aim of this study was to investigate whether catechins formulations, containing 20% and 35% (−) forms (2S, 3R), added to a high-fat diet, may inhibit the development of atherosclerosis in apoE mice more effectively than the native catechins.

2. Materials and methods

2.1. Preparing catechins formulations and their composition analysis

The green tea Polyphenon 60 from green tea (Sigma–Aldrich) was used. The catechins were used in the native form (A) and thermally modified (B, C). 5% of aqueous solutions of catechins were thermally modified. The process was carried out in anaerobic conditions. The aqueous solutions were bubbled with nitrogen gas for 2 min and placed in Scott's test tubes. The modification was performed at the temperature of 140 °C for 40 min (B) and 80 min (C).

The content and the type of catechins in the formulations were determined by a high-performance liquid chromatography (HPLC) as described by Lina et al. [16]. A liquid chromatography, spectrophotometric detector, $\lambda = 280$ nm and an LUNA C18(2) (250 × 4.6 mm) column were used for the above. Samples were eluted from the column following the programme: 0–10 min–100% of phase A –isocratic elution; 10–25 min–100%–90% of phase A, 0%–10% of phase B – linear gradient; 25–60 min–90%–70% of phase A, 10%–30% of phase B – linear gradient. The flow rate was 1 ml/min. Phase A comprised methanol, formic acid and redistilled water (20:0.3:79.7 v/v/v) and phase B composed of methanol and formic acid (99.7:0.3 v/v).

2.2. Animal and diets

Homozygous 10-week-old male apolipoprotein E knockout mice (apoE-knockout) were purchased from Taconic Laboratory (Taconic Europe A/S, Denmark). The mice were divided into five groups ($n = 7$). Each group of mice was kept in a separate cage and housed in an air-conditioned room (21 °C), with 12 h light/dark cycles and the humidity at 55 ± 10%. Food and water were provided *ad libitum*. For the first two weeks the mice were being acclimated to the experimental conditions. At this time, the animals were fed with commercial pellets diet for rodents. After the acclimatization period, the mice were fed AIN-93 modified diets for 12 weeks [17]. A standard diet (DS) containing 52.95% cornstarch, 20% casein, 10% sucrose, 7% oil, 5% fibre, 3.5% mineral mix (AIN-93G-MX), 1% vitamin mix (AIN-93-VX), 0.3% L-cystine, 0.25% choline as well as a high-fat diet (HFA, HFB, HFC) containing 39.95% cornstarch, 20% casein, 10% sucrose, 19,9% butter, 5% fibre, 3.5% mineral mix (AIN-93G-MX), 1% vitamin mix (AIN-93-VX), 0.3% L-cystine, 0.25% choline and 0.09% catechins formulations (A, B, C). Total energy of the standard diet and high-fat diet was estimated as 3948 kcal/kg and 4589 kcal/kg. The estimate of caloric content was based on the standard physiological fuel values for protein, fat, and carbohydrate of 4, 9, and 4 kcal/g respectively. Animals fed standard diet (DS) were the reference group, whereas the control group was the mice fed high-fat diet (HF) without catechins. Diets were divided into the single daily portions and stored at the temperature of −20 °C. Before feeding the portions were mixed with water at a ratio of 2: 1. All uneaten food was removed, dried at 60 °C and weighed. Mice were weighed weekly. Two days before ending the experiment, the blood glucose taken from the tail vein was determined. At the end of the experiment, the mice were injected with heparin and after 10 min were anesthetized with pentobarbital (40 mg/kg of the body weight). The blood samples from the abdominal aorta were placed in tubes. The plasma samples were separated by centrifugation 12 000 × g for 2 min and stored at the temperature of −80 °C until the analysis. The organs were washed with PBS by direct injection in the heart left ventricle. The heart and the aorta were collected from the animals.

The research involving animals was approved: No. 78/VI/2009 dated 16.06.2009, issued by the Local Ethics Committee for Experiments on Animals in Cracow.

2.3. Determination of plasma antioxidative activity

The concentration of lipid peroxidation products reacting with 2-thiobarbituric acid (TBARS) in animals plasma was determined spectrophotometrically method using a commercially available kits (Cell Biolabs, Inc.). TBARS are expressed as μmol of malondialdehyde (MDA).

2.4. Determination of plasma triglycerides and cholesterol levels

The concentration of triglycerides (TG), total cholesterol (Chol-T), cholesterol associated with high density lipoprotein (HDL-C) and cholesterol associated with low density lipoprotein (LDL-C) in mice's plasma were determined by an enzymatic method using a commercially available kits (Cormay).

2.5. Quantification of atherosclerosis in aortas by en face analysis

In anesthetized mice, the thorax was longitudinally opened, the right atrium was incised, and the heart was perfused with phosphate-buffered saline (PBS, pH 7.4) through the apex of the left ventricle. The aorta from arch to bifurcation was dissected out from surrounding tissues and fixed in 4% paraformaldehyde. Then, it was opened longitudinally, pinned onto brown silicone plates, and stained with Sudan IV (Sigma–Aldrich, St. Louis, MO, USA). The aortic lesion area and total aortic area were measured using the LSM Image Browser.

2.6. Quantification of atherosclerosis in aortic roots by cross-section analysis

The heart and the ascending aorta were dissected. The excised heart and ascending aorta were embedded in OCT compound (CellPath) and snap-frozen. 10 μm-thick cryosections were cut from the aortic root using a standardized protocol [18,19]. Eight sections were collected at 100-μm intervals starting at a 100-μm distance from the appearance of the aortic valves. Sections were thaw-mounted on poly-L-lysine coated slides and air dried. After fixation in 4% paraformaldehyde (pH = 7), sections were stained with Meyer's hematoxylin and oil red-O (Sigma–Aldrich). Oil red-O-stained sections were examined under an Olympus BX50 microscope and used for quantitative evaluation. Images of the aorta were recorded using an Olympus Camedia 5050 digital camera and stored as TIFF files of resolution 1024 × 768 pixels. The total area of the lesion was measured semiautomatically in each slide using LSM Image Browser 3 software. For each animal a mean lesion area was

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