

DRUG BIOCHEMISTRY

POLYPHENOLS ACTION AGAINST OXIDATIVE STRESS FORMATION
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Abstract: The aim of this study was to investigate the influence of epigallocatechin-3-gallate (EGCG), theaflavins (TFs) and black tea extract (BTE) on oxidative stress formation as well as on antioxidant system of human vein endothelial cells (HUVEC). HUVEC were incubated for 0,5 h with 100 mM *tert*-butyl hydroperoxide (*t*-BHP) for oxidative stress formation. The influence of EGCG, TFs, and BTE on oxidative stress and antioxidant system parameters was investigated by the pre-incubation for 2 h with 50 mg/mL of each compound. Half hour exposure to *t*-BHP caused statistically significant decrease in GSH-Px activity and in the content of GSH, vitamin A, vitamin E as well as tryptophan. Moreover, pretreatment of cells with *t*-BHP caused statistically significant increase in activities of Cu,Zn-SOD, GSSG-R and in the level of MDA and dityrosine. Pretreated with *t*-BHP endothelial cells, subjected to EGCG, TFs and black tea extract, are partially protected against oxidative activity of *t*-BHP causing statistically significant increase in GSH-Px activity, GSH and tryptophan level and decrease in MDA and dityrosine level in comparison with HUVEC pretreated with *t*-BHP group. These results indicate the beneficial effect of tea polyphenolic compounds on HUVEC antioxidant abilities and, in consequence, their protective effect in cell components.

Keywords: antioxidant system, black tea, epigallocatechin gallate, oxidative stress

Oxygen, essential to aerobic life can be toxic when generation of reactive oxygen species (ROS) is uncontrolled. ROS are formed mainly during oxygen reduction in mitochondrial electron transport chain. They may be also formed during the cyclooxygenase pathway, as well as during reactions catalyzed by cellular enzymes such as cytochrome P450 oxidase and xanthine oxidase (1). The superoxide radicals are generated as the first ones and they are subsequently converted to hydrogen peroxide in univalent reduction through nonenzymatic or enzymatic dismutation. Independently, the superoxide radicals play also an important role in organism defense, since activated phagocytes generate ROS to fight with invading microorganisms. They are also induced by a wide variety of stimuli, including many xenobiotics and cytokines. However, the organism is protected by a tight regulation of production and elimination of ROS. The primary defenses against ROS are enzymatic and

nonenzymatic antioxidants. An imbalance in the production and elimination of ROS may lead to oxidative stress. In such situation, ROS easily react with lipids, proteins and nucleic acids oxidatively modifying their structure and function (1).

Irrespective of endogenous antioxidant system, their action is assisted by exogenous antioxidants, especially those originating from natural products. One kind of such potentially health promoting beverages is tea. It is generally believed that green tea prepared by dehydration of *Camellia sinensis* leaves containing monomeric polyphenols – catechins, possesses antioxidant properties (2, 3). Recent investigations, however, have indicated that black tea obtained by tea leaves fermentation contains smaller amount of catechins, though more multimeric polyphenols – theaflavins and thearubigins. Biological activities of those extracts reveals antioxidant abilities, though they are less documented, but still extensively examined (4-6).

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Endothelial cells make the first line of defense for all tissues. They are also the first targets of oxidative stress in such conditions as hypoxia or ischemia-reperfusion (7). It has been shown that endothelial cells often contain less antioxidant enzymes than other cells, like fibroblasts, type II epithelial cells or smooth muscle cells (8, 9). In the blood vessels, endothelial cells are more sensitive to oxygen than smooth cells and than adventitial fibroblasts. Since endothelial cells occur in every vascularised tumor, they appear to be an important target for antioxidative substances. A comparison of the effect of black tea extract and the most important tea polyphenols on oxidative stress formation in endothelial cells makes this approach applicable for understanding the pathological processes which take part, e.g. in cancer development.

The objective of the present study has been to investigate the potentially protective effect of epigallocatechin gallate [EGCG], theaflavins [TFs] and black tea extract against oxidative stress induced by *tert*-butyl hydroperoxide (*t*-BHP) in human umbilical vein endothelial cells (HUVEC). In order to evaluate oxidative stress, the activity of antioxidant enzymes such as SOD, GSH-Px, GSSG-R and reduced glutathione – GSH, vitamin A and vitamin E level and oxidative stress markers such as MDA, dihydroxyacetone and tryptophan level were measured.

EXPERIMENTAL

Materials and method

Human umbilical vein endothelial cells [HUVEC] were isolated from human umbilical cord veins by 0.1% collagenase solution. They were incubated in the water bath at the temperature of 37°C for 10 min and perfused with the HBSS solution. The eluate was centrifuged for 10 min (1000 × g) and HUVEC sedimentation was suspended in the tissue culture medium M199, and placed into fibronectin coated cell culture flasks (75 cm²) (Nalge Nunc International, USA). The M199 tissue culture medium was supplemented with 20% fetal bovine serum, 100 U/mL penicillin, 100 µg/mL streptomycin, 0,25 µg/mL amphotericin B, 100 µg/mL heparine, 200 µg/mL endothelial cell growth factor and 25 mM HEPES buffer. The incubation was done in 5% CO₂ and 95% room air (Heraeus incubator, Thermo Electron Corporation, USA). The medium was changed every other day. Cells from the 4th passage were used in the 5-fold repetitions. The HUVEC cultures were identified by differential immunocytochemical staining to confirm their purity. More than 95% of cells were stained positively

with antibody von Willebrand factor. The Ethics Committee of the Medical University of Białystok approved the experiments.

The cell culture flasks containing HUVEC were divided into the following groups:

- HUVEC – non-stimulated HUVEC constituted the control culture.
- HUVEC + EGCG – HUVEC incubated for 2 h with 50 mg/mL EGCG (Sigma – Aldrich, USA).
- HUVEC + TFs – HUVEC incubated for 2 h with 50 mg/mL theaflavins TFs (Sigma – Aldrich, USA).
- HUVEC + black tea – HUVEC incubated for 2 h with 50 mg/mL black tea extract (standard research blends – lyophilized extract; TJ Lipton, Englewood Cliffs, NJ). Black tea extract contained both the catechins: epigallocatechin gallate (EGCG) – 14.53 mg/L, epigallocatechin (EGC) – 2.21 mg/L, epicatechin (EC) – 2.83 mg/L, and the theaflavins (TFs): theaflavin (TF₁), theaflavin 3-gallate (TF_{2A}), theaflavin 3'-gallate (TF_{2B}), theaflavin 3, 3'-gallate (TF₃) in the amount of 156,16 mg/g of dried extract. The levels of catechins and theaflavins were determined by modified HPLC methods of Mattila (10) and Lee (11).
- HUVEC + *t*-BHP – HUVEC incubated for 0,5 h with 100 mM *tert*-butyl hydroperoxide.
- HUVEC + *t*-BHP + EGCG – HUVEC pre-incubated for 2 h with 50 mg/mL EGCG and co-incubated for 0,5 h with 100 mM *tert*-butyl hydroperoxide.
- HUVEC + *t*-BHP + TFs – HUVEC pre-incubated for 2 h with 50 mg/mL TFs and co-incubated for 0,5 h with 100 mM *tert*-butyl hydroperoxide.
- HUVEC + *t*-BHP + black tea – HUVEC pre-incubated for 2 h with 50 mg/mL black tea extract and co-incubated for 0,5 h with 100 mM *tert*-butyl hydroperoxide.

After incubations, HUVEC were washed with PBS, suspended in the lysing buffer and disintegrated by means of freezing at the temperature of -70°C and thawed under tap water. Cells were centrifuged (10000 × g /4°C/10 min) and samples of supernatant were taken for analysis.

Biochemical assays

Superoxide dismutase (SOD; EC.1.15.1.1) activity was determined by the method of Misra and Fridovich (12) modified by Sykes (13). This method measures the activity of cytosolic superoxide dismutase. One unit of SOD activity was defined as the amount of the enzyme required to inhibit the oxidation of epinephrine to adrenochrome by 50%. The

enzyme activity was expressed in units per milligram protein.

Glutathione peroxidase (EC.1.11.1.6) activity was measured spectrophotometrically using the method of Paglia and Valentine (14). GSSG formation was assayed by measuring the conversion of nicotinamide adenine dinucleotide phosphate (NADPH) to NADP. One activity unit was defined as the amount of enzyme catalyzing conversion of 1 mmol of NADPH/min per mg protein at 25°C and pH 7.4.

Glutathione reductase (EC.1.6.4.2) activity was measured spectrophotometrically using the method of Mize and Langdon (16), which monitors the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm. The enzyme activity was expressed in units per mg of protein.

The GSH concentration was obtained from subtraction of the GSSG value from the total glutathione value. Total glutathione was determined by HPLC technique (16). A reduction of the disulfide group of oxidized glutathione with dithiothreitol was essential for this assay. The main step of total glutathione determination is based on the derivatization of GSH with *o*-phthalaldehyde, which reacts with both the sulfhydryl and the primary amino group of glutathione to form a highly fluorescent product. The oxidized glutathione was measured by HPLC method after elimination of GSH with *N*-ethylmaleimide followed by reduction of disulfides with dithiothreitol and derivatization with *o*-phthalaldehyde (17).

The vitamins A and E were extracted from cell lysate with hexane containing 0,025% butylated hydroxytoluene. The hexane phase was removed and evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in 100 μ L of methanol and 50 μ L was injected on RP 18 column (5 μ m, 125 \times 4 mm). The HPLC separation was carried by isocratic elution (mixture 95% methanol and 5% water) with spectrophotometric detection at 294 nm (18). The obtained retention time for vitamin A and E was: 1,87 min and 4,75 min, respectively.

Lipid peroxidation was assayed by HPLC measurement of malondialdehyde (MDA) as a malondialdehyde-thiobarbituric acid adducts by HPLC with spectrofluorometric detection (excitation λ = 532 nm, emission λ = 553 nm) (19). The procedure involved protein precipitation step, acidic hydrolysis and adducts with thiobarbituric acid (TBA) formation. The HPLC separation of MDA-TBA adducts was performed with the mobile phase consisted of 40:60 (v/v) 0.05 M methanol-potassium phosphate buffer, pH 6.8, on RP C18 column.

Protein oxidative modifications were examined

by dityrosine and tryptophan content. Tryptophan and dityrosine were measured with a spectrofluorometer Hitachi 2500. Signal intensity was calibrated against 0.1 mg/mL quinine sulfate solution in sulfuric acid which fluorescence was assumed as a unit. Dityrosine content was estimated by fluorescence spectrophotometry at 325 nm for excitation and 420 nm for emission (20). Fluorescence emission at 338 nm and excitation at 288 nm were used to measure tryptophan content (20).

The protein concentration was determined according to Lowry et al. (21).

Statistical analysis

Data obtained in the current study are expressed as the mean \pm SD. These data were analyzed by standard statistical procedures: one-way analysis of variance (ANOVA) with Tukey test for multiple comparisons, to determine significance between different groups. A *p* value of < 0.05 was considered significant.

RESULTS

HUVEC treatment with EGCG, TFs and black tea extract did not show statistically significant changes in superoxide dismutase activity. Statistically significant increase, in comparison with HUVEC group (by about 13%), was observed in Cu,Zn-SOD activity when HUVEC was treated with 100 mM *tert*-butyl hydroperoxide (Tab 1). Moreover, statistically significant changes in Cu,Zn-SOD activity were not reported after treatment of HUVEC with *t*-BHP and the examined antioxidants in comparison with HUVEC treated only with *t*-BHP.

Activity of glutathione peroxidase was observed to be increased after treatment of HUVEC with TFs and black tea extract (by about 15% and 19%, respectively). Statistically significant decrease in the activity of GSH-Px (by about 58%) was noticed in HUVEC treated with *t*-BHP in comparison with HUVEC group (Tab. 1). However, the activity of glutathione peroxidase was statistically increased in case of TFs and black tea extract by about 32% and 42%, respectively, after pretreatment of endothelial cells with *t*-BHP and co-incubation with antioxidants.

It was shown that treating HUVEC with EGCG and black tea extract caused statistically significant increase in glutathione reductase, by about 15% and 30%, respectively, in comparison to HUVEC group (Tab. 1). However, treating HUVEC with *t*-BHP

caused statistically significant increase in GSSG-R activity (by about 26%) when compared to HUVEC group. Co-incubation of HUVEC (treated earlier with *t*-BHP) with black tea extract caused an increase in glutathione reductase activity in comparison with HUVEC as well as HUVEC + *t*-BHP group.

These results have shown statistically significant increase in the level of GSH in groups of HUVEC treated with *t*-BHP (by about 30%) (Tab. 1). The statistically significant increase in GSH level was also observed after HUVEC incubation with *t*-BHP and with antioxidants (by about 17%, 20% and 28% for EGCG, TFs and black tea extract, respectively).

The statistically significant decrease in the level of nonenzymatic antioxidants – vitamin A and E, was observed after HUVEC exposure to *t*-BHP (about 20% and 17% for vitamin E and vitamin A, respectively) (Tab. 1). It was also observed that co-incubation of pretreated with *t*-BHP endothelial cells with exogenous antioxidants (EGCG, TFs or black tea extract) caused an increase in the level of the examined vitamins in comparison with the group treated only with *t*-BHP.

Significant changes were also reported in the HUVEC level of lipid peroxidation marker – malondialdehyde (MDA) (Tab. 2). HUVEC treated with TFs and black tea extract revealed statistically significant decrease in MDA level (by about 8% and 18% for TFs and black tea extract, respectively). An exposure of HUVEC to *tert*-butyl hydroperoxide

caused statistically significant increase (by about 59%) in MDA content. However, after treatment of endothelial cells with *t*-BHP and co-incubation with TFs and black tea extract, statistically significant decrease in MDA level was observed (by about 19% and 37% for TFs and black tea extract, respectively) in comparison with HUVEC treated only with *t*-BHP.

Moreover, it was also proved that the examined antioxidants caused statistically significant changes in protein oxidative modification. Changes in the level of protein dityrosine and tryptophan indicate an occurrence of this process. In case of HUVEC treated with EGCG, TFs as well as black tea extract, the level of dityrosine was increased by about 19%, 15% and 9%, respectively (Tab. 2). However, statistically significant decrease in tryptophan content was also observed (by about 8%, 12% and 16% for EGCG, TFs and black tea extract, respectively) in comparison with HUVEC group. Statistically significant changes were observed after HUVEC exposure to *t*-BHP (dityrosine – an increase by about 42%, tryptophan – a decrease by about 30%) when compared with HUVEC group. Nevertheless, all examined antioxidants caused statistically significant changes in the marker of protein oxidation content after co-incubation with HUVEC pretreated with *t*-BHP (dityrosine – an increase by about 11%, 27% and 35%, tryptophan – a decrease by about 11%, 20% and 24% for EGCG, TFs and black tea extract, respectively).

DISCUSSION

Table 1. Antioxidant enzymes activity and non-enzymatic antioxidants level in the HUVEC after incubation with epigallocatechin gallate [EGCG], theaflavins [TFs] and black tea extract, as well as after treatment with *t*-BHP and incubation with EGCG, TFs and black tea extract. Data are presented as the mean \pm SD; n = 5.

Examined group protein]	SOD [U/mg protein]	GSH-Px [mU/mg protein]	GSSG-R [mU/mg protein]	GSH [nmol/mg protein]	Vit E [nmol/mg protein]	Vit A [pmol/mg]
HUVEC	2.02 \pm 0.12	564 \pm 37	3.54 \pm 0.21	174 \pm 10	17.6 \pm 1.1	23.9 \pm 1.5
HUVEC + EGCG	2.22 \pm 0.14	589 \pm 35	4.18 \pm 0.23 ^a	183 \pm 10	16.8 \pm 1.0	24.5 \pm 1.5
HUVEC + TFs	2.29 \pm 0.16	662 \pm 41 ^a	3.76 \pm 0.21	187 \pm 10	17.2 \pm 1.0	24.2 \pm 1.5
HUVEC + black tea	2.32 \pm 0.19	694 \pm 40 ^a	5.04 \pm 0.34 ^a	196 \pm 20	17.8 \pm 1.1	23.7 \pm 1.3
HUVEC + <i>t</i> -BHP	2.47 \pm 0.14 ^a	237 \pm 18 ^a	4.78 \pm 0.31 ^a	122 \pm 10 ^a	14.2 \pm 0.9 ^a	19.7 \pm 1.1 ^a
HUVEC + <i>t</i> -BHP + EGCG	2.52 \pm 0.17 ^a	267 \pm 17 ^{a,b}	4.96 \pm 0.35 ^{a,b}	148 \pm 10 ^{a,b,c}	14.8 \pm 0.9 ^c	21.0 \pm 1.1 ^b
HUVEC + <i>t</i> -BHP + TFs	2.76 \pm 0.20 ^{a,b,c}	348 \pm 21 ^{a,c,e}	5.02 \pm 0.36 ^{a,c}	153 \pm 10 ^{a,c,e}	15.1 \pm 1.0 ^a	21.8 \pm 1.2
HUVEC + <i>t</i> -BHP + black tea	2.59 \pm 0.18 ^a	411 \pm 25 ^{a,d,e}	5.57 \pm 0.36 ^{a,c}	169 \pm 20 ^{d,e}	15.9 \pm 1.1	22.0 \pm 1.2

a – significantly different from HUVEC group ($p < 0.05$); b – significantly different from EGCG group ($p < 0.05$); c – significantly different from TFs group ($p < 0.05$); d – significantly different from black tea group ($p < 0.05$); e – significantly different from *t*-BHP group ($p < 0.05$)

Table 2. Level of lipid peroxidation product – malondialdehyde [MDA] and markers of protein oxidative modifications [dityrosine and tryptophan] after incubation of HUVEC with epigallocatechin gallate [EGCG] theaflavins [TFs] and black tea extract as well as after treatment with *t*-BHP and incubation with EGCG, theaflavins and black tea extract. Data are presented as the mean \pm SD; n = 5.

Examined group	MDA [pmol/mg protein]	Dityrosine [U/g protein]	Tryptophan [U/g protein]
HUVEC	36.2 \pm 2.0	0.90 \pm 0.05	2.94 \pm 0.16
HUVEC+EGCG	34.5 \pm 2.1	0.73 \pm 0.03 ^a	3.19 \pm 0.16 ^a
HUVEC+TFs	33.1 \pm 2.0 ^a	0.76 \pm 0.03 ^a	3.35 \pm 0.17 ^a
HUVEC+black tea	29.7 \pm 1.8 ^a	0.82 \pm 0.04 ^a	3.52 \pm 0.19 ^a
HUVEC+ <i>t</i> -BHP	86.6 \pm 5.6 ^a	1.56 \pm 0.10 ^a	2.07 \pm 0.11 ^a
HUVEC+ <i>t</i> -BHP+EGCG	79.5 \pm 4.4 ^{a,b}	1.39 \pm 0.08 ^{a,b,c}	2.34 \pm 0.12 ^{a,b,c}
HUVEC+ <i>t</i> -BHP+TFs	70.3 \pm 4.2 ^{a,c,c}	1.13 \pm 0.06 ^{a,c,c}	2.56 \pm 0.14 ^{a,c,c}
HUVEC+ <i>t</i> -BHP+black tea	54.5 \pm 3.5 ^{a,d,e}	1.02 \pm 0.06 ^{a,d,e}	2.7 2 \pm 0.17 ^{d,e}

a – significantly different from HUVEC group ($p < 0.05$); b – significantly different from EGCG group ($p < 0.05$); c – significantly different from TFs group ($p < 0.05$); d – significantly different from black tea group ($p < 0.05$); e – significantly different from *t*-BHP group ($p < 0.05$)

Dietary antioxidants, including polyphenolic compounds, are considered beneficial because of their protective role in the pathogenesis of multiple diseases such as cancer, coronary heart disease and atherosclerosis associated with oxidative stress formation. One of the polyphenolic compound, which is commonly found in various foods, is epigallocatechin gallate (EGCG). This is the major antioxidant of tea leaves and green tea (22). The main antioxidant constituents of black tea are multimeric polyphenols – theaflavins and in smaller amount – catechins, including EGCG (23). Each of polyphenolic black tea components is characterized by strong antioxidant properties but it is difficult to predict the effectiveness of their mixture because the synergistic action of polyphenols contained in green tea has been revealed (24). Moreover, it is not clear whether they act only as antioxidants or also as oxidants, because, for example, EGCG and green tea show prooxidative properties at high concentrations (25).

This study demonstrates that EGCG, theaflavins and black tea, have the ability to partially protect the human umbilical vein endothelial cells against oxidative insult by modulating antioxidative abilities. *t*-BHP, often applied as a model compound to induce oxidative stress in cell systems by producing free radical intermediates (26), has also been used in the present study. There are two distinct pathways of *t*-BHP metabolism; one *via* cytochrome P450, and the other by glutathione peroxidase converting *t*-BHP to *tert*-butanol and oxidized GSH (27,

28). These metabolic pathways could increase cellular ROS (27), which may attack phospholipids, proteins, and nucleic acids. This is confirmed by results of the present study which indicate that proteins as well as lipids were oxidatively modified after cells treatment with *t*-BHP.

It is known that major targets for all ROS are proteins. All proteins are susceptible to attack by ROS, but some of them are more vulnerable than the others (29). Moreover, from among amino acids composing protein moiety the most sensitive to oxidation are sulfhydryl and aromatic amino acids. This study has proved that after *t*-BHP administration the concentration of tryptophan was significantly decreased, whereas the level of dityrosine – the product of ROS reaction with tyrosine, was increased. It had been shown that tryptophan residues undergo destruction in reactions with different reactive species generated oxidative stress, such as singlet oxygen hydrogen peroxide or *t*-BHP (30-32). During oxidative stress formation the different derivatives of tryptophan were identified: hydroperoxides, alcohols, diols, N-formylkynurenine, kynurenine, 2-, 4-, 5-, 6-hydroxytryptophan, oxindole-3-alanine and its derivatives, but also a decrease in tryptophan fluorescence was observed as a consequence of tryptophan residue structure changes (32, 33). Dityrosine production appears to be a useful “marker” for protein modification, especially by hydroxyl radical (34). Modifications of individual amino acids residues may result in changes in secondary and tertiary structure of pro-

teins and in consequence in protein functions (35). Independently of protein modifications caused directly by ROS, protein structure can also be changed by reactions with lipids oxidative modification products generated in consequence of *t*-BHP metabolism. A variety of compounds, which could be produced during lipid peroxidation, may exert toxic effects on cells, but the most reactive are malondialdehyde and 4-hydroxynonenal (36). That property can be attributed to their structure, which gives these biogenic aldehydes strong electrophilic properties and reflects the ability to form adducts with nucleophilic sulfhydryl, amino and histidyl groups of proteins, which cause changes in proteins structure and function. It was proved that 4-hydroxynonenal inhibits glutathione peroxidase in this way (37). Glutathione peroxidase activity inhibition is additionally enhanced by specific reactions between malondialdehyde or 4-hydroxynonenal and selenocysteine residue of active center of glutathione peroxidase (38, 39). Its expression depends on the availability of selenium compounds that are able to act as precursors of selenoproteins biosynthesis and on GSH level that is GSH-Px cofactor. All elements, protein moiety, selenium and GSH needed for activity of GSH-Px may be disturbed by aldehydes generated during lipid peroxidation. Because GSH-Px catalyses detoxifications of peroxides including lipid peroxides, the diminution of its activity observed in this study suggests that it may not be sufficiently efficient in reacting with peroxides generated in the region of the membrane (40). Peroxidation process after *t*-BHP treatment is also intensified by a decrease in the levels of vitamins A and E, which act as lipophilic antioxidants and cooperate together in membrane protection from ROS.

While glutathione peroxidase activity is decreased after treatment of endothelial cells with *t*-BHP, the activity of superoxide dismutase and glutathione reductase is increased. It contributes to enhanced dismutation of superoxide anions and higher level of hydrogen peroxide. It was shown that oxidants, including hydrogen peroxide, may activate gene expression through the antioxidant responsive elements (ARE) *via* electrophilic thiol modification and such expression was observed in human fibroblasts (41). On the other hand, an increase in GSSG-R activity is likely to result from the tendency of the cells to maintain proper GSH level (42). However, GSH level significantly decreases at the same time. In such a situation the increase in the activity of GSSG-R, which controls the endogenous level of GSH – GSH-Px coenzyme, does not improve antioxidant action of glutathione peroxidase, which

raises the level of lipid peroxidation products.

These findings are very important because human endothelial cells have been shown to be more susceptible to oxidative stress than fibroblasts which results from substantial differences in the make-up of their antioxidant defence system (43). In endothelial cells the glutathione redox-cycle plays a predominant role as an antioxidant defenses against peroxides in general (44). It is extremely important because of their location at the interface of the vascular system. Endothelial cells in the blood vessel wall may be exposed to peroxides, for instance, during local inflammatory reactions or contact with oxidized lipoproteins (45). Therefore, an imbalance in this system may dramatically contribute to the onset of endothelial cell senescence in various pathological conditions related to vascular diseases and chronic inflammations.

Thus, stabilizing endogenous antioxidant systems is very important for normal function of endothelial cells and more attention has been recently paid to taking advantage of natural antioxidants found in the fruits, vegetables or beverages supplied to the organism with everyday diet (46). Tea, which similar to water is the most popular drink in the world, is one of the beverages characterized by antioxidative properties. The present study has proved that black tea and, to a smaller degree, its components enhance antioxidant properties of endothelial cells. Black tea components with early proved antioxidant abilities are catechins, especially EGCG. This compound possesses the ability to prevent oxygen radicals formation *via* inhibiting activity of enzymes participating in their generation, and to scavenge the free radicals as well as to chelate transition metal ions which enhance radical reactions (20). Recent examinations have also proved that the main multimeric polyphenols of black tea – theaflavins generated during tea leaves fermentation possess even stronger antioxidant abilities than their precursors – catechins (47). It has been found that theaflavins possess the ability to prevent oxygen radicals formation, *via* inhibiting activity of xanthine oxidase – the enzyme participating in superoxide anion generation (48). Moreover, theaflavins are able to scavenge the superoxide anion, singlet oxygen, hydroxyl radical and they react with radicals many times faster than strong antioxidants (49). It may explain the observed in this study improved glutathione peroxidase activity as well as GSH and vitamins level increased especially after theaflavins and black tea extract application.

Application of polyphenolic antioxidants can cause a decrease in free radical level as well as in radicals ability to react with proteins and phospho-

lipids, resulting in prevention of their oxidative modifications. EGCG, theaflavins and black tea scavenge free radicals in the aqueous phase as well as in the lipid bilayer. It is known that catechins and, to a higher degree, theaflavins show partially lipophilic character and can preferentially enter the hydrophobic core of the membrane, where they exert a membrane stabilizing effect by modifying the lipid packing order and cause a dramatic decrease in lipid fluidity in this region of the membrane (50). Polyphenols can also interact with phospholipid head groups, particularly with those containing hydroxyl groups, so they can also decrease the fluidity in the polar surface of phospholipid bilayer (51). Their localization in the membrane interior and their influence on fluidity of membrane components can sterically hinder diffusion of free radicals and thereby decrease the kinetics of free radical reactions. Moreover, the above polyphenols influence antioxidant capacity in biomembranes (52). Catechins prevent also the consumption of lipophilic antioxidant – α -tocopherol, by repairing tocopheroxyl radical and protection of the hydrophilic antioxidant – ascorbate, which also repairs this radical (53). In such a way they also decrease the lipid peroxidation when membrane phospholipids are exposed to free radicals from the aqueous phase. The oxidative attack from the aqueous phase seems to be an important reaction for initiating membrane lipid peroxidation.

CONCLUSIONS

In summary, we showed that tea polyphenolic compounds have beneficial effect on HUVEC antioxidant abilities and in consequence their protective effect in cell components. Independently of the fact that endothelial cells are the first line of defense for all the tissues, they are also components of every vascularized tumor. Therefore, they seem to be an important target for oxidative agents as well as antioxidants such as polyphenols. Protective action of polyphenols helpful in physiological conditions intensifies the disease during cancer development. It is especially important in the case of polyphenols of tea that is consumed by a majority of the world population.

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