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THE EFFECT OF QUERCETIN, CHLOROGENIC ACID AND EPIGALLOCATECHIN ON PROLIFERATION OF CACO-2 CELLS

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Abstract. Dietary polyphenols are considered beneficial because of their potential protective role in the pathogenesis of chronic diseases associated to oxidative stress. However, many of these effects may depend on the concentration of the antioxidants used. The aim of the study was to evaluate the effect of quercetin, chlorogenic acid and epigallocatechin on proliferation of human colon adenocarcinoma cells Caco-2. Caco-2 cells were treated with different concentrations (25, 50, 100, 200, and 400 μM in DMSO) of antioxidants and the proliferation was assayed by MTT method. The results of the study indicated that chlorogenic acid and quercetin enhanced Caco-2 cells proliferation in time- and concentration-dependent manner. In contrary, epigallocatechin exhibit inhibitory influence, particularly significant at concentration ≥ 200 μM.

Key words: human colon adenocarcinoma Caco-2 cells, oxidative stress, antioxidants, polyphenols, proliferation

INTRODUCTION

Oxidative stress is the consequence of an imbalance of prooxidants and antioxidants in the organism and is linked with pathogenesis of many chronic diseases [Baer-Dubowska et al. 2007]. Epidemiological studies have led to the conclusion that diet containing an abundance of fruit and vegetables are protective against a variety of diseases, particularly cardiovascular disease and cancer [Heber 2004]. It is mainly due to antioxidant compounds that are present in fruit and vegetables [Heber and Bowerman 2001]. Polyphenols are secondary plant metabolites, which exhibit a wide range of biological effects as a consequence of their antioxidant properties. Quercetin, chlorogenic acid, epigallocatechin are the main representatives of different polyphenol classes

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present in the diet. Quercetin is an aglycone of many flavonols common in berries, onion, cabbages and lettuces. Chlorogenic acid is one of the hydrocinnamic acids. The main source of chlorogenic acid is coffee, but is also present in apples, pears, and plums. Epigallocatechin is one of the main tea catechin monomers and belongs to flavanols [Manach et al. 2004]. Polyphenols inhibit LDL oxidation [Frankel et al. 1993, Ivanov et al. 2001] and protect DNA from oxidative damage [Cook and Samman 1996, Gackowski and Oliński 2007]. Moreover, polyphenols have antitoxin and antimicrobial properties [Friedman 2007]. Polyphenols show anticarcinogenic and antimutagenic effects as they may inactivate carcinogens, inhibit the expression of mutant genes and activate enzymatic systems involved in the detoxification of xenobiotics [Bravo 1998]. However, some polyphenols have been reported to be detrimental. Several studies have shown that some antioxidants exhibit prooxidant activity under certain conditions (particularly in the presence of transition metal ions) and may cause oxidative DNA degradation [Sakihama et al. 2002, Azam et al. 2004, Labieniec and Gabryelak 2006]. Many of these effects may depend on the concentration of the polyphenol utilized since high doses of some phenolic compounds may be prooxidant and negatively affect cell growth and viability [Agullo et al. 1994, Raza and John 2005]. Some researches postulate that this prooxidant action of plant polyphenols may be an important mechanism of their anticancer properties [Yang et al. 1998, Azam et al. 2004, Lee et al. 2005, Bhat et al. 2007].

Caco-2 cells were isolated from a primary colonic tumour in a 72-year-old Caucasian male and constitute one of the most utilized *in vitro* models of colon cancer [Rösmann et al. 2002, Lee et al. 2005]. The aim of the study was to evaluate the effect of different concentrations of quercetin, chlorogenic acid, and epigallocatechin on the proliferation rate of Caco-2 cells.

MATERIALS AND METHODS

Chemicals. A human colon adenocarcinoma cell line Caco-2 (ATCC No. HTB-37) was purchased from LGC Promochem (UK). Quercetin dihydrate, (-)epigallocatechin from green tea, chlorogenic acid, Eagle's Minimum Essential Medium (EMEM), Dulbecco's Modified Eagle's Medium (DMEM), Non-essential Amino Acids (NEAA), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), trypsin, Phosphate Buffered Saline (PBS), dimethyl sulfoxide (DMSO), Fetal Bovine Serum (FBS), L-glutamine, antibiotics were obtained from Sigma-Aldrich (Poznań, Poland). Isopropanol (analytical grade) was purchased from POCh Company (Gliwice, Poland).

Cell culture. Frozen Caco-2 cells were resuscitated and proliferated in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% FBS, 1% L-glutamine, 1% NEAA and 1% antibiotics. The cells were maintained for 48 h in controlled conditions (37°C, 98% of humidity, 5% CO_2, Nuaire, Plymouth, USA) up to reaching confluence. At confluence, i.e. when monolayer was formed, cells were washed in PBS (without Ca^{2+}/Mg^{2+}), harvested by treatment with 0.25% trypsin and centrifuged at 200 × g for 15 min. Then the cells pellet was resuspended in fresh growth medium of reduced FBS content (5%). All experiments were performed with Caco-2 cells at least of 70% confluence (1×10^9 cells × cm^{-3} of medium). To assess the influence of antioxidants on Caco-2, cells were treated with different concentrations of chlorogenic acid, quercetin and epigallocatechin dissolved in dimethyl sulfoxide (DMSO). The control cells were

treated with DMSO only. Experiments were performed in triplicate and the results were shown as an arithmetic mean.

Cell proliferation. The MTT Assay was used to measure the cell proliferation rate. Cells were seeded into 96-well plates (Corning, NY, USA) and exposed to antioxidants (at final concentrations of 25, 50, 100, 200, and 400 μM). After 24 and 48 hours the medium was removed from above cells and 0.1 cm^3 of MTT in DMEM (0.5 mg × cm^{-3}) was added to each well. The cells were incubated for 4 hours (37°C, 5% of CO_2) until purple precipitate was visible. The MTT formazan formed was solubilized with 1 cm^3 acidic isopropanol (0.04 M HCl in absolute isopropanol) by incubating at room temperature in the dark for 2 hours on the horizontal shaker. Absorbance of the converted dye was measured at a wavelength of 570 nm (PowerWave XS, Biotek, Vermont, USA). The results were expressed as percentages of the control value.

Cells count. Cells were grown to confluence in 6-well plates, then treated with antioxidants solutions (at final concentration of 25, 50, 100, and 400 μM), and after 24 or 48 h washed with PBS to remove unattached cells. Methylene blue was used for cell staining and clarity of counting. Caco-2 cells were counted under light microscope (Nikon TMS, Nikon Instruments Inc., Melville, NY, USA). Eight different fields of view were examined in each well and the results expressed as mean cells number per field of view.

RESULTS AND DISCUSSION

Cells proliferation is an important process that enables regeneration and turnover of epithelial cells in gut, but uncontrolled proliferation might be dangerous and lead to tumours initiation. On the other hand, the ability to limit the proliferation rate of tumour cells is very valuable feature of chemical compounds used as medicaments. We showed that used polyphenols had different effect on human colon adenocarcinoma Caco-2 cells. Cells number was dependent on the concentration of polyphenols used and the time of exposition (Table 1). The results of the study indicated that chlorogenic acid and quercetin had activated Caco-2 cells proliferation. The effect was time- and concentration-dependent. Chlorogenic acid at low doses (25-50 μM) was more potent stimulator (Fig. 1), while at higher concentrations quercetin was better. In contrary, epigallocatechin exhibited inhibitory influence on Caco-2 cells proliferation, particularly significant at concentration ≥ 200 μM. Many authors demonstrated that antioxidants at concentrations lower than 100 μM have antiproliferative influence on different cancer cell lines. This was demonstrated for quercetin against HepG2 cells [Granado-Serrano et al. 2006]; human breast cancer cell line [Roy et al. 2005] and gastric carcinoma cell lines (MKN-1, MKN-45, MKN-74, and KATO-III) [Horie et al. 2005] were affected by epigallocatechin-3-gallate, and green tea polyphenols influenced human lung adenocarcinoma cell lines (NCI-H661, NCI-H441 and NCIH1299) and human colon cancer cell line (HCT-116, HT-29) [Yang et al. 1998, Woude et al. 2003]. In our research only epigallocatechin suppressed proliferation. We suppose, this activity was caused by prooxidant action of epigallocatechin. It was demonstrated that some antioxidants such as gallic acid, catechins and epigallocatechin-3-gallate can induce H_2O_2 generation in culture medium [Yang et al. 1998, Lee et al. 2005, Furukawa et al. 2003]. Hydrogen peroxide subsequently causes DNA oxidation and exerts strong antiproliferative and proapoptotic effect, which can be abolished by the treatment with catalase.

Table 1. The number of Caco-2 cells in cultures exposed to quercetin (Q), chlorogenic acid (ChlA) and epigallocatechin (EGC) at final concentration of 25-400 μM. The results represent the mean cells number ±SD/field of view (n = 8)

	Control	Antioxidant	25 μM	50 μM	100 μM	200 μM	400 μM
24 h	11.1 ±0.8	Q	12.9 ±0.6	15.4 ±1.2	16.1 ±1.9	27.3 ±1.9	41.2 ±7.6
		ChlA	19.8 ±1.1	19.9 ±1.4	20.8 ±0.8	22.7 ±0.9	27.7 ±2.7
		EGC	8.7 ±0.9	9.3 ±1.1	10.6 ±1.5	5.5 ±1.6	2.9 ±0.9
48 h	19.4 ±1.1	Q	21.9 ±0.9	24.5 ±1.3	46.3 ±2.1	87.1 ±3.4	119.0 ±8.9
		ChlA	51.8 ±2.1	49.6 ±2.5	45.0 ±5.1	82.4 ±6.9	74.3 ±6.8
		EGC	29.4 ±3.4	21.0 ±2.9	17.3 ±4.6	4.9 ±2.3	2.3 ±1.1

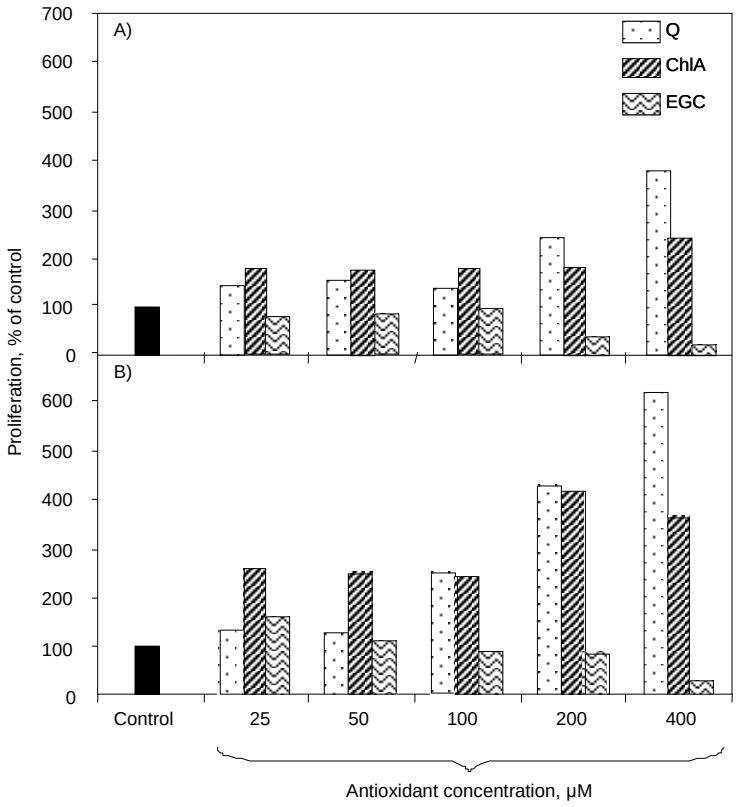

Fig. 1. The effect of quercetin (Q), chlorogenic acid (ChlA) and epigallocatechin (EGC) on Caco-2 cells proliferation. Cells were exposed on antioxidants in the range of concentration from 25 to 400 mM for 24 h (A) and 48 h (B). Proliferation was determined by MTT method and expressed as percentage of control cells (not exposed). The results represents arithmetic mean from 3 independent experiments

The different impact of antioxidant used in our study may be caused not only by different chemical structure of those polyphenols. It was proved that the antioxidants effect is dependent on their concentration and a status of the cells. Agullo et al. [1994] showed that quercetin (in the range of concentration from 15 μM to 120 μM) exerted a preferential cytotoxic effect on active proliferating Caco-2 and HT-29 cells, and failed to affect cell viability in confluent cells. The differences between normal and cancer cells sensitivity on antioxidants were also shown by Yamamoto et al. [2003]. Epigallocatechin-3--gallate induced oxidative stress only in cancer cells favouring their destruction without affecting normal cells. For the colon carcinoma cell lines HT-29 and HCT-116, at relatively high concentrations of quercetin, a significant decrease in cell proliferation was proved. However, at lower concentrations, a subtle but significant stimulation of cell proliferation was observed [Woude et al. 2003]. In our *in vitro* model we used Caco-2 cells at confluence. Hence, we suppose that upon reaching confluence the cells expressed morphological and biochemical features of adult human enterocytes and therefore should be treated as *in vitro* model of normal human small intestinal epithelium and not of cancer cells. Otherwise, the results showing the quercetin- and chlorogenic acid-induced stimulation of cell proliferation are worrying. As those polyphenols are abundant in the daily diet they may promote the tumour growth. The detailed analysis with their bioavailability taken into consideration should be realized.

It should be mentioned that the methodological differences in culture conditions between laboratories may influence the expression of morphological and functional characteristics of cells and lead to selection of sub-populations of cells becoming prominent in the culture [Sambuy et al. 2005]. Culture-related conditions, as well as the different Caco-2 cell lines utilized in different laboratories, often make extremely difficult to compare results in the literature and show the need to standardize and optimize of this intestinal model.

CONCLUSIONS

We conclude that Caco-2 cells should be carefully used as *in vitro* model in studies on human colon cancer. In our model cells react rather like normal intestinal cells than cancer cell line. Quercetin and chlorogenic acid at the concentration range of 25-400 μM stimulated Caco-2 cell proliferation, while epigallocatechin suppressed it.

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WPŁYW KWERCETYNY, KWASU CHLOROGENOWEGO I EPIGALOKATECHINY NA PROLIFERACJĘ KOMÓREK CACO-2

Streszczenie. Polifenole zawarte w diecie uważa się za pożyteczne ze względu na ich potencjalnie ochronną rolę w patogenezie przewlekłych chorób wywołanych przez stres oksydacyjny. Jednakże, wiele z obserwowanych efektów może zależeć od stężenia użytych przeciwutleniaczy. Celem badań była ocena wpływu kwercetyny, kwasu chlorogenowego i epigalokatechiny na komórki Caco-2. Proliferację komórek Caco-2 poddanych działaniu różnych stężeń przeciwutleniaczy (25, 50, 100, 200 i 400 μM w DMSO) oceniano w teście z MTT. Wykazano, że kwas chlorogenowy i kwercetyna zwiększały proliferację komórek Caco-2 w stopniu zależnym od czasu i stężenia. Natomiast epigalokatechina wykazywała hamujący wpływ, szczególnie w stężeniu ≥ 200 μM.

Słowa kluczowe: komórki Caco-2, stres oksydacyjny, przeciwutleniacze, polifenole, proliferacja

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