RESEARCH ARTICLE

Antioxidants May Protect Cancer Cells from Apoptosis Signals and Enhance Cell Viability

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Abstract

Quercetin is one of the most abundant dietary flavonoids widely present in many fruits and vegetables. Previous in vitro studies has shown that quercetin acts as an antioxidant and anti-inflammatory agent and it has potent anticarcinogenic properties as an apoptosis inducer. In this study we examined apoptotic effects of quercetin on the K562 erythroleukemia cell line. K562 cells were induced to undergo apoptosis by hydrogen peroxide. Cell viability and apoptosis level were assessed by annexin V and PI staining methods using flow cytometry. Viability of K562 cells was increased by low dose of quercetin (5-100 µM) for 3 hours. High doses of quercetin proved toxic (100-500 µM, 24 hours) and resulted in decrease of K562 cell viability as expected ($p<0.01$). As to results, 100 µM quercetin was defined as a protective dose. Also, K562 cell apoptosis due to hydrogen peroxide was decreased in a dose dependent manner. As indicated in previous studies, reduction of superoxides by free radical scavengers like quercetin could be beneficial for prevention of cancer but consumption of such flavonoids during cancer treatment may weaken effects of chemotherapeutics and radiotherapy. Especially cancer patients should be carefully considered for traditional phytotherapy during cancer treatment, which can lead to controversial results.

Keywords: Quercetin - cancer - apoptosis - prevention - cell viability

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Introduction

Reactive oxygen species (ROS) are highly reactive molecules generated predominantly during cellular respiration and normal metabolism. These side products can damage many biological molecules; they can change protein functions, damage DNA material and cause lipid peroxidation of cell membranes (Kulbacka et al., 2009). In general, the reducing environment inside cells helps to prevent free radical mediated damage. This reducing environment is maintained by the action of antioxidant enzymes and substances, such as superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione, vitamins C and E (Bayir, 2005; Schafer et al., 2001).

Oxidative stress can be result of dysbalance between the production of free radicals and antioxidant system which plays a role in the pathogenesis of fatal diseases such as cancer and cardiovascular diseases (Kopani et al., 2006). Excessive production of ROS may lead to programmed cell death and may accelerate the process of aging (Chamond et al., 1999).

For a long time, reactive oxygen species (ROS) have been considered harmful mediators of inflammation owing to their highly reactive nature. However, there are an increasing number of findings suggesting that ROS can play role in anti-inflammatory and prevent autoimmune responses (Hultqvist et al., 2009).

Neutralization of free radical is important to prevent the damage of cells, tissues and organs. Control of free radical neutralizations are under the control of internal antioxidant enzymes such as SOD, NADH, NADPH (Kopani et al., 2006). Destruction of balance between the oxidant and antioxidants depends on age, environmental conditions and some mutations. Uses of herbal sourced ingredients to improve balance of antioxidant system are common way, between the public.

Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin (Formica and Regelson, 1995). Surface OH groups of flavonoids such as Quercetin, tends to get relationship with reactive or free oxygen species. It has been reported that some flavonoids such as rutin (quercetin-3-rutinoside) and quercetin shows antioxidant activity via this way (Ueno et al., 1984).

Quercetin is one of the most abundant dietary flavonoids. It can be found in apples, black, green and buckwheat tea, onions, raspberries, red grapes, cherries, citrus fruits as well as in some well known medical plants (ginkgo biloba, cranberries and St John's wort) (Hertog and Hollman, 1996; Terao, 2009).

In previous *in vitro* studies has shown that quercetin acts as an antioxidant and anti-inflammatory agent and that it has potent anticarcinogenic properties as apoptosis inductor.

In the normal conditions, herbal sourced antioxidants

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intake could be beneficial for cancer prevention (Gerhauser, 2008; Murakami et al., 2008), coronary artery disease prevention (Siu, 2010) and moderation of aging process. But what will happen if cancer patient takes artificial herbal sourced antioxidants such as quercetin which is abundantly present in many herbs, fruits and grains. Will it facilitate anticancer therapy or not?

To examine that, K562 erythroleukemia cell line was induced to apoptosis by hydrogen peroxide and effect of quercetin over the apoptosis and viability of K562 cells was observed with flow cytometer.

Materials and Methods

Cell culture

K562 cells (Erythroleukemia cell line derived from a chronic myeloid leukemia patient in blast crisis) were incubated in the RPMI 1640 medium (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) containing 10% FCS and penicillin/streptomycin (Gibco-Invitrogen Ltd., Paisley, Scotland) in the CO_2 incubator (37ºC, 5%CO_2). Cell count was made with tyrpan blue. Viability of K562 cells were maintained between 85-90%.

Apoptosis induction by quercetin

Effect of quercetin (Sigma-Aldrich, St. Louis, MO, USA) on the cell viability of K562 was evaluated with different Quercetin concentration (5-500 μM) through 3 hours with Flow cytometer (Becton-Dickinson, San Jose, CA, USA). Toxic dose of Quercetin concentrations (100-500 μM) was evaluated through 24 hours.

Apoptosis induction by hydrogen peroxide

K562 cells were induced to apoptosis by with hydrogen peroxide (H_2O_2 Riedel de Haen, D3016, Seelzel, Germany) in different concentration (150, 300, 600 μM) and protective effect of Quercetin (100 μM) was analyzed through 24 hours. For this aim, protective dose of Quercetin (100 μM) was added on to K562 cell (106 cells in 10 mL RPMI 1640, 10% FCS, penicillin/streptomycin), then hydrogen peroxide were added in to RPMI 1640 medium.

FITC-Annexin V staining assay

After induction with H_2O_2 and Quercetin, K562 cells were washed twice with PBS and suspended in 1x binding buffer (10 mM HEPES, 140 mM NaCl, and 5 mM $CaCl_2$ at pH 7.4) at a concentration of approx 1×10^5 cells/ml. Five µl of FITC-Annexin V and 10 µl of propidium iodide (Sigma, A2214, 1;100) was added to both control and induced cell suspension from the 50 µg/ml stock. After incubation at room temperature for 10 minutes at dark, the fluorescence of the cells was determined immediately with a flow cytometer (FACS Calibur, Becton-Dickinson, San Jose, CA, USA).

Statistical analysis

Measurements were repeated more than 3 times and student t-test was used for statistical analysis. Descriptive statistics for studied variables (characteristics) were presented as mean, standard deviation, minimum and maximum values. Two ways Factorial ANOVA was used to compare Groups. Duncan multiple comparison test was used to determine different groups. Statistical significance levels were considered as 1% ($p<0.01$). The SPSS (ver. 13) statistical program was used for all statistical computations.

Results

For the time-course and dose-response experiments, K562 erythroleukemia cells were treated with 5, 10, 20, 40, 80, 100, 200, 300, 400 and 500 µM of quercetin for 3. Cell viability was assessed by Annexin V/PI flow cytometer assay. Figure 1 shows that quercetin (In the 5-200 μM doses for 3 hours) caused slight increase of K562 cells viability (Figure 1) but high doses of quercetin (over than 200 μM) caused to increase of apoptotic cell death ($p<0.01$).

For second experiment K562 cells were treated with 100, 200, 300, 400 and 500 μM of quercetin for 24 h. Toxic doses of quercetin (doses higher than 100 μM, 24 hours) resulted with decrease of K562 cell viability as expected ($p<0.01$) (Figure 2). Thus, 50-100 μM of quercetin was determined as the cell protective quercetin dose range.

In third probe, hydrogen peroxide (H_2O_2) was applied in dose 150, 300 and 600 μM for 24h. Kind of ROS, hydrogen peroxide (H_2O_2) induced apoptosis of K562 cells had been shown previously and similar results was obtained in our study ($p<0.01$) (Figure 3, Table 1).

To determine preventive role of quercetin (free radical scavenger flavonoid) against to H_2O_2 (ROS) induced

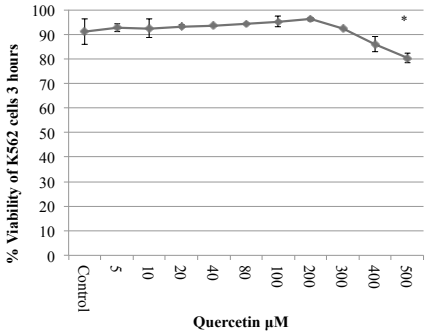

Figure 1. Effect of Quercetin Over the Viability of K562 Cells for 3 Hours. Non-apoptotic cell rate (% viable cells) was assessed by FITC-Annexin V/PI flow cytometer assay. *p<0.05

Table 1. Descriptive Statistics and Comparison Results for Groups

H_2O_2	Control				H_2O_2				H_2O_2+Quercetin 100 μM			
	Mean	St. Dev.	Min.	Max.	Mean	St. Dev.	Min.	Max.	Mean	St. Dev.	Min.	Max.
150	76.77*,**	8.01	71.7	86	67.07*,**	10.16	61.2	78.8	73.93*,**	7.51	69.6	82.6
300	76.77*,**	8.01	71.7	86	45.90*,**	8.29	37.2	53.7	68.30*,**	7.79	62.7	77.2
600	76.77*,**	8.01	71.7	86	29.20*,**	10.85	19.3	40.8	59.33*,**	10.41	51.3	71.1

*Different lower case represent differences among HP level ($p<0.01$); **Different upper case represent differences among groups ($p<0.01$)

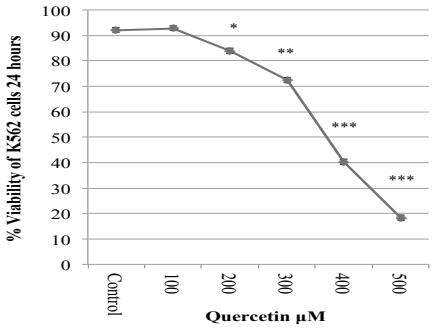

Figure 2. Effect of Quercetin Toxic Doses Over the K562 Cells Viability for 24 Hours. Non-apoptotic cells rate (% viable cells) was assessed by FITC-Annexin V/PI flow cytometer assay. *p<0.05, **p<0.01, ***p<0.001

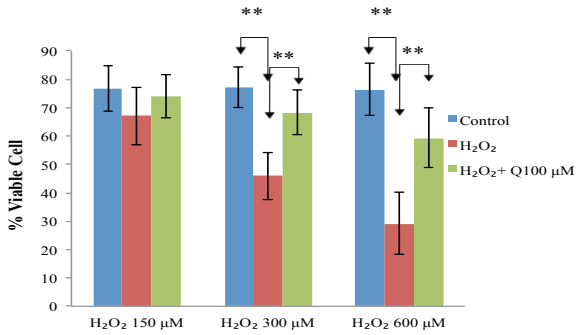

Figure 3. Protective Effect of Quercetin Over the H_2O_2 Induced Apoptosis of K562 Cell Line for 24 Hours. Non-apoptotic cell rate (% viable cells) was assessed by FITC-Annexin V/PI flow cytometer assay. **p<0.01

apoptosis, 100 μM of quercetin was added into cell culture medium (106 cell in the 10 ml RPMI 1640 include 10% FCS, penicillin-streptomicin), 20 minute before the 150, 300, 600 μM H_2O_2 treatment. K562 cells viability were maintained by the Quercetin from the H_2O_2 induced apoptosis (p<0.01), (Figure 3, Table 1).

Discussion

In previous studies quercetin was implied as an apoptotic activator and antioxidant as well as protective agent against various types of cancer (Jung et al., 2010). It was shown that quercetin can inhibit proliferation of tumor cells and reduce the number of aberrant crypt foci in colon tumors (Van Erk et al., 2005) that it can facilitate programmed cell death in lung carcinoma (Nguyen et al., 2004) and colonorectal tumor cells (Richter et al., 1999). Other researchers had showed that quercetin acts as a free radical scavenger and protects cells from oxidizier molecules (Orsolic et al., 2007; Benkovic et al., 2009). As known, oxidative stress and free radicals are important activators for apoptosis (Rinaldi et al., 2009).

On the other hand, studies conducted in last ten years showed that quercetin can act as an antiapoptotic agent as well (Ishikawa and Kitamura, 2000). In a few studies it has been shown that quercetin can partially prevent H_2O_2 - induced apoptosis (Chow et al., 2005) and it was suggested that protective effects of quercetin against oxidative injuries of some cells may be achieved via modulation of mitochondrial dysfunction and inhibition of caspase activity (Park et al., 2003).

In this study, our results showed that higher dose of quercetin than 200 µM reduce K562 cell viability (which considered as toxic doses by Cao et al. (2007) but low doses of quercetin (<200 μM) can increase cell viability and considered as therapeutic dose. As it known, hydrogen peroxide is an important apoptotic mediator over mitochondrial dependent apoptotic mechanism; we also showed protective effect of quercetin over the K562 cells which induced to apoptosis with H_2O_2.

Free radical generation and inactivation's are under the strict control of oxidoreductive reactions. Excessive superoxide production can accelerate cell death, DNA damage and can lead to cancer, also excessive inactivation of superoxides can disturb apoptosis signals. Inactivation of radiation and environmental sourced superoxides by the herb sourced flavonoid free radical scavengers seems beneficial for cancer prevention (Murakami et al., 2008) but if cancer is already present, as known as free radicals are important for apoptosis signals. For this reason, uncontrolled consumption of flavonoid free radical scavengers such as quercetin during chemothrapy may weaken the effect of chemotherapeutics and radiotherapy rather then help it.

It has been theorized that cancer risk reduction may be achieved by greater consumption of phytochemical-rich fruits and vegetables (Davis et al., 2009; Rinaldi et al., 2009). However, results of our research suggest that antioxidants can interfere and attenuate success of anticancer therapy so antioxidant intakes should be strictly controlled in the cancer diagnosed patients.

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