Modulatory effect of quercetin on DNA damage, induced by etoposide in bone marrow cells and on changes in the activity of antioxidant enzymes in rats

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Abstract

The aim of the studies was to evaluate the influence of quercetin on etoposide-induced DNA damage in rat bone marrow cells and on changes in the activities of superoxide dismutase (SOD), catalase and antioxidant power in lung tissue (AOP). Quercetin was administrated by gavage at a dose of 135 mg/kg b.w. for three consecutive days, and one hour after the last dose, etoposide was intraperitoneally injected, at dose of 80 mg/kg b.w. Bone marrow cells were harvested from the femurs 24 hours later and DNA damage was evaluated by the comet assay, under alkaline condition. The study provided evidence that quercetin, administered to rats, provides major protection against etoposide-induced DNA damage in bone marrow cells and does not influence either the SOD activity, lowered by etoposide, or AOP, enhanced by etoposide.

Introduction

One of the most important questions in chemotherapy regards the possibility of DNA damage prevention in healthy bone marrow cells during treatment with etoposide, without lowering the antitumour potency of the drug. We approached this issue by checking the effect of etoposide treatment, followed by quercetin administration, on DNA in bone marrow cells of healthy rats. The rationale behind that attempt was such that quercetin may prevent oxidation of etoposide phenoxyl radicals, generated by myeloperoxidase, a constitutively expressed enzyme, the activity of which is particularly high in myeloid progenitor cells [1-3]. Such an antioxidant recycling may enhance the antioxidative potential [2]. However, quercetin can react with etoposide only if it is still in circulation. Because the maximum quercetin concentration occurs within up to 1 h after its intake, at least, in human studies [4], the etoposide was administered after 1 h from the last quercetin exposure. A significant decrease in DNA damage in bone marrow cells was observed 24 h later. Those results confirmed our previous observation with stimulated human lymphocytes, which also showed that quercetin, given together with etoposide, lowered the extent of DNA damage [5].

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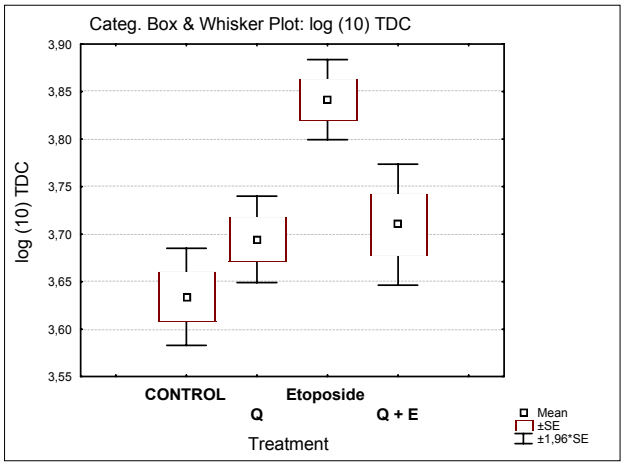
Material and Methods

Quercetine was administered by gavage at final concentration of 135 mg/kg b.w. to two groups of rats (three-month-old male Wistar rats -WAG/Krf), every 24 hours for three consecutive days. On the third day, 1 h after the last dose of quercetin, the first group (designated in all figures and tables as "quercetin") was sacrificed. At the same time, the etoposide, at a dose of 80 mg/kg b.m., was intraperitoneally given to the second group of rats, pretreated with quercetin (designated in all figures and tables as "quercetin +etoposide". The third group of rats received only etoposide (designate in all figures and tables as"etoposide"). After 24 h from the exposure to etoposide, each individual was sacrificed. The lungs were removed from all the rats in each group and stored at - 20^0 C. Bone marrow cells were flushed from femurs into PBS (Ca^{2+}, Mg^{2+} free) and gently resuspended in a mixture, containing dimethylsulphoxide (DMSO) and fetal bovine serum (FBS), to obtain final concentrations of DMSO at 10% v/v and FBS at 50% v/v. The cellular mixture was cooled at approximately 10C per min. and stored at -80^0C until analysis was performed (within less than two weeks). The control rats were also sacrificed at that time. The activity of SOD was measured by the Misry and Fridovich

Table 1. The activity of SOD, catalyse and a ferric-reduction antioxidant power (FRAP) was measured in the homogenate of lung tissue isolated from the control group of rats (1) and 24 hours after: (2) last dose (135 mg/kg b.w.) of quercetin; (3) etoposide (80 mg/kg b.w.); (4) etoposide treatment 1 h after last quercetin administration. Post-hoc test (one-way ANOVA) was performed and differences (P - values) between groups (means) are presented.

treatment SOD activity	[1]	[2]	[3]	[4]
control [1]		0.576	0.066	0.163
quercetin [2]	0.576		0.014	0.061
etoposide [3]	0.066	0.014		0.925
quercetin+etoposide [4]	0.164	0.0614	0.925	
treatment catalase activity	[1]	[2]	[3]	[4]
control [1]		0.853	0.918	0.448
quercetin [2]	0.853		0.916	0.522
etoposide [3]	0.918	0.916		0.448
quercetin+etoposide [4]	0.448	0.522	0.448	
treatment antioxidant power	[1]	[2]	[3]	[4]
control [1]		0.812	0.009	0.012
quercetin [2]	0.812		0.009	0.013
etoposide [3]	0.009	0.009		0.537
quercetin+etoposide [4]	0.012	0.013	0.537	

Figure 1. The mean values of DNA damage (TDC) measured by comet assay in bone marrow cells isolated from control rats and isolated 24 hours after: a) the last dose (135mg/kg b.w.) of quercetin administrated for three consecutive days; b) etoposide (80mg/kg b.w.); c) etoposide treatment given 1 h after the last dose of quercetin (135mg/kg b.w.) administration.



method [6] and catalase activity was evaluated by the Aebi assay [7]. The ferric-reducing antioxidant power (FRAP) was determined in homogenate of lung tissue by the Bezni and Strain method [8].

The comet assay was performed, according to the original laboratory protocol for the application of the pH>13 alkaline single cell gel assay, to detect DNA damage in the cells [9]. Electrophoresis was conducted at 0.74 1V/cm for 30 min. The current was adjusted to 300 mA. An image analysis system (CometPlus from ThetaSystem GmbH, Germany) was used for the quantification of DNA damage. The percentage of DNA in the tail (TDC) was automatically generated. At least two slides per one rat, with 50 randomly selected cells per slide, were analyzed. The one-way ANOVA and post-hoc test comparison of the means was performed to detect differences between the groups (means) of data from the FRAP, SOD and catalase activity tests. The data, obtained from the comet test (TDC), were analyzed by the same test, following log transformation. The experiments were performed in accordance with Polish legal requirements and under a license, granted by the Commission of Bioethics of the Jagiellonian University.

Results and Discussion

The amount of endogenous alkali-labile DNA damages, analyzed by the comet assay in bone marrow cells, was not changed by quercetin administration (P= 0.09), whereas etoposide treatment induced highly significant DNA damage (P = 0.000000). Ouercetin, administered before etoposide treatment, drastically lowered the amount of DNA strand breaks, as compared to the values, when etoposide was given alone (P=0.0008). That amount was non-significantly different from the damages, observed in bone marrow cells after quercetin administration alone (P=0.7) (Fig. 1).

It is evident from Table 1, that quercetin, administered alone, slightly increased the activities of both antioxidant enzyme, as SOD, and catalase. The same trend was observed with regard to the AOP, measured by the FRAP assay. However, the statistical analyses revealed that that enhancement was not significantly different from the control values.

The effects of etoposide administration were evaluated after 24 h. As shown in Table 1, etoposide treatment did not influence catalase activity but decreased the SOD activity only at the borderline of statistical significance (P = 0.066). In spite of that, AOP was significantly higher (P = 0.009), as compared to the respective value in the control rats. When etoposide injection was given 1 h after the last quercetin dose and lung tissue was

removed 24 h later, the catalyze activity remained unchanged, as compared to: the control (P=0.918), etoposide (P= 0.448) and quercetin given alone (P=0.522). SOD activity continued to be the same, as compared with that, when etoposide was given alone (P=0.925), being at the borderline of statistical significance (P= 0.0614), when compared with respective values, when quercetin was given alone. The AOP after treatment with both compounds was still significantly higher than that in the control (P=0.012) and after quercetin alone (P=0.013), but it did not significantly differ (P=0.537) from the values, observed in etoposide treatment.

The presented data provide evidence that a prolonged exposure to quercetin does not affect either SOD or catalase activities. That what was also shown by Breinholt et all [10]. Similarly, Hilbert et al showed [11] that no significant down regulation of SOD or CAT was observed in the lung lavage cells, despite an increased concentration of vitamin E and beta carotene. The lowered SOD activity in etoposide-treated rats is opposed to expected results if SOD was to be important for DNA damage protection. An analogous result was obtained after bleomycin treatment. Two days after bleomycin administration, total lung SOD, CAT, and GSHP activities were significantly depressed - between 15 and 25%. [12].

Effects of prolonged administration of quercetin, causing the decrease in genotoxicity of etoposide treatment, may indicate that nutritional strategies for preventing or ameliorating the DNA damage in normal cells is possible.

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