

**QUERCETIN INFLUENCES PHOSPHOLIPASE A2
PRODUCTS IN A549 ADENOCARCINOMA CELLS**

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Abstract

The effect of quercetin on phospholipase A2 activity and its products has been investigated in A549 adenocarcinoma cells. Quercetin is a polyphenol of medico-biological significance, which has been reported to show antioxidant and anti-tumour beneficial effects. Phospholipids are functionally active molecules, which participate in various cellular processes, acting as second messengers, enzyme activators, membrane transporters, etc. Although there are many studies dealing with the influence of quercetin on the lipid metabolism of different types of cancer cells, the mechanism of this effect remains largely unclear. The present study showed that quercetin treatment of A549 cells induced activation of phospholipase A2 (PLA2) activity, the latter being known as a source of pro-inflammatory intermediate metabolites. The phospholipid analysis of membranes from A549 cells revealed reduction of phosphatidylcholine (PC) and an increase of the level of lysophosphatidylcholine (LPC). In addition, acyl chain analysis showed an elevation of some polyunsaturated fatty acids, such as arachidonic acid (AA), the latter being a major product of PLA2. The content of lipid peroxides was also elevated in membranes from quercetin-treated A549 cells, which we presume is related to the higher content of polyunsaturated fatty acids, which are an excellent target of oxidative destruction.

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In conclusion, the present results show that treatment of A549 adenocarcinoma cells with the polyphenol quercetin induces activation of membrane-bound PLA2, which is a source polyunsaturated fatty acids, the latter producing lipid peroxides. We presume that this mechanism underlies the reported by other authors elevation of oxidative stress, which is induced by quercetin treatment of cancerous cells.

Key words: quercetin, phosphatidylcholine, phospholipase A2, arachidonic acid, lipid peroxides

Introduction. Quercetin (3,3',4',5,7-pentahydroxyflavone) is a polyphenolic compound that is widely distributed in certain vegetables and fruits. It is associated with antioxidant, anti-inflammatory and even anti-tumour effects [1-3]. It has been reported to induce apoptosis in both A549 and H1299 human lung cancer cells [4]. Numerous investigations are devoted to the beneficial health effects of quercetin in various pathologies such as oxidative stress, toxicological stress after pesticide intake, neurodegenerative disorders, inflammation, neoplastic processes, age-related diseases, etc. [5]. In addition, there is evidence indicating that flavonoids take part in regeneration of certain antioxidant molecules [6]. However, the molecular mechanisms underlying the effects of quercetin and the possibilities for its implication in the pharmaceutical practice still require additional clarification.

In our previous studies we analyzed the effect of quercetin on three-dimensional fibroblast cultures in order to test its effect on healthy non-cancerous cells grown in *in vivo*-like conditions [7]. The obtained results showed that quercetin treatment induced changes in the lipid metabolism, down-regulation of the pro-inflammatory PLA2 and augmentation of the relative content of saturated fatty acids in the membrane phospholipids. In addition, a reduction in the lipid peroxide level was observed, which was an expected result of cell treatment with antioxidants.

The aim of the present study was to investigate the effect of quercetin treatment on the lipid metabolism of lung adenocarcinoma A549 cells and more specifically on the mechanism of accumulation of free fatty acids, the latter being an excellent target of oxidative damage and a source of lipid peroxides.

Materials and methods. Treatment of cells with quercetin was performed as explained elsewhere [8]. Plasma membranes from the analyzed cells were isolated as described earlier [9]. Lipid extraction was performed according to BLIGH and DYER [10]. Phospholipids were determined according the procedure of KAHOVCOVA and ODAVIC [11]. Phospholipase A2 activity was performed by the method described elsewhere [12] with slight modifications. Plasma membranes were incubated with 100 nmol egg yolk phosphatidylcholine as a substrate in 100 mmol/L Tris-HCl pH 8.6 with 5 mmol/L CaCl₂ and 0.1% fatty acid free bovine serum albumin. The reaction was stopped with 0.5 mL methanol and the liberated fatty acids were extracted. For fatty acid analysis the extracted phospholipids were saponified with 0.5 N methanolic KOH and methylated with boron trifluoride

ethanol complex (Merck) [13]. The fatty acid methyl esters were extracted with hexane and separated by gas chromatography on a capillary column Supelcowax 10. The level of lipid peroxides was determined by ELISA Lipid Peroxidation kit, Abcam, as described in the manufacturer instruction.

Results. Treatment of A549 adenocarcinoma lung cells with the polyphenol quercetin induced decrease of the level of the major membrane phospholipid – PC. Table 1 shows that the membrane level of PC was reduced, whereas the content of LPC was elevated due to quercetin treatment. Since PC is a substrate and LPC is a product of PLA2, we measured the changes of PLA2 activity induced by quercetin treatment of A549 cells. Figure 1 shows that incubation with quercetin caused a statistically significant activation of PLA2 in the cancerous cell line, which implies that this process most probably underlies the observed alterations in the membrane lipid composition (Table 1). PLA2 is known to influence the

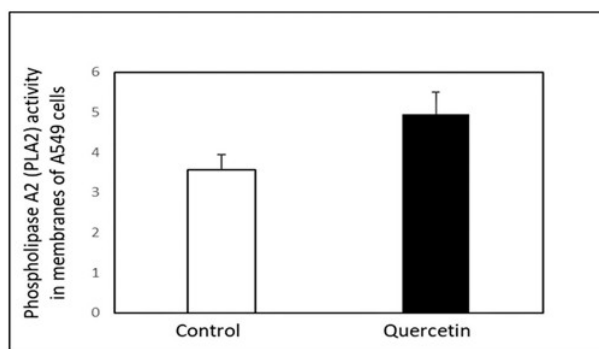


Fig. 1. Phospholipase A2 specific activity, expressed as nmol/min/mg protein in membranes of A549 cells treated with quercetin. All experiments were performed in triplicates. Differences are statistically significant $P < 0.01$

T a b l e 1

Phospholipid composition of plasma membranes from A549 cells treated with quercetin ($\mu\text{g}/\text{mg}$ protein)

Lipids	Control	Quercetin
Lysophosphatidylcholine	1.8	2.7*
Sphingomyelin	8.4	6.8*
Phosphatidylcholine	127.4	109.2*
Phosphatidylserine	7.9	8.6
Phosphatidylinositol	10.5	11.1
Phosphatidylethanolamine	25.3	27.1*
Phosphatidylglycerol	10.9	10.3

* $P < 0.01$

T a b l e 2

Fatty acid composition of membrane phospholipids from A549 cells treated with quercetin (mol%)

Fatty acids	Control	Quercetin
C16:0	37.7	33.6*
C18:0	18.3	15.8*
C18:1	15.1	18.2*
C18:2	13.6	15.1*
C20:4	8.8	11.2*
C22:4	6.5	6.1

* $P < 0.01$

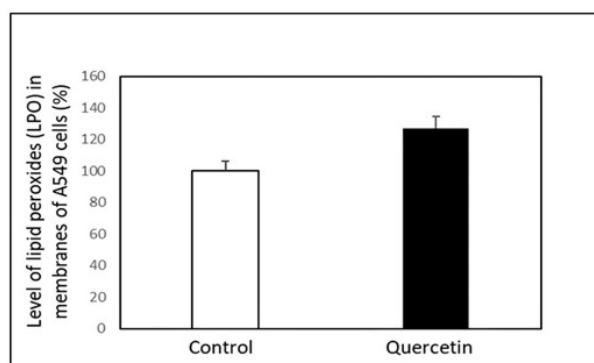


Fig. 2. Alterations in the level of lipid peroxides in membranes of A549 cells treated with quercetin. Values are expressed as % of controls (100%). All experiments were performed in triplicates. Differences are statistically significant $P < 0.001$

fatty acid composition of membrane phospholipids, because it is responsible for deacylation of the phospholipid molecules at *sn*2 position. Thus, we analyzed the alterations in the fatty acid composition of the membranes associated with the activated PLA2. As evident from Table 2, quercetin treatment induced elevation of the relative content of unsaturated fatty acids and especially of arachidonic acid (AA), which is a major acyl chain product of PLA2. In addition, since unsaturated fatty acids are a perfect target of oxidative attack and a potent source of lipid peroxides, we further evaluated the changes in the level of lipid peroxides in the membranes of cancer cells, treated with quercetin. The results showed that the content of lipid peroxides was elevated by 27% as a result of quercetin treatment, implying that quercetin induced augmentation of the oxidative stress in the treated tumour cells (Fig. 2).

Discussion. Quercetin is a flavonoid and is one of the most important and widely used dietary antioxidants. It is found in different vegetables and fruits, tea, and various kinds of food supplements. Quercetin has been reported to possess antioxidant, anti-inflammatory and immunomodulatory effects and has attracted much research attention in recent years because of its anticancer effects [14–16].

In the present study we analyzed the changes in the phospholipid metabolism, induced by quercetin treatment of human adenocarcinoma A549 lung cells. The analysis of the membrane lipid composition showed that the level of PC was decreased due to quercetin treatment (Table 1). In our previous studies we analyzed the influence of quercetin on the level of membrane glycerophospholipids as a result of treatment of three-dimensional fibroblast cell cultures, the latter being considered a model approximating closely living tissues [7]. In these studies we observed an elevation of PC and a decrease of LPC which seem to be controversial to the data reported in the present work. We suggest that these differences are due to the opposite effect displayed by quercetin on cancer and non-cancer cells. In healthy normal fibroblasts quercetin effect was associated with increase of membrane PC and a reduction of LPC and these changes were in agreement with the alterations observed for PLA2. PLA2 activity is related to maintenance of the acyl chain composition of the membrane phospholipids [12]. Basically, this enzyme hydrolyzes the acyl chains found in *sn*2 position in the phospholipid molecules. These acyl chains are mostly unsaturated, which makes PLA2 an enzyme, providing the membranes with unsaturated free fatty acids and also supplying the cells with precursors of the pro-inflammatory leucotrienes, thromboxanes and prostaglandins, which act as mediators in the processes of inflammation [17]. Thus, it seems likely, that the activation of PLA2 as a result as quercetin treatment of the lung tumour cells is associated with elevation of the pro-inflammatory intermediate metabolites. To test this presumption we measured the alterations in the level of the saturated and polyunsaturated fatty acids in order to analyze the differences in the phospholipid composition induced by quercetin treatment of the cancer cells. The changes in the acyl chains of the membrane phospholipids and the ratio between saturated and polyunsaturated fatty acids are important for maintenance of the physico-chemical properties of membranes which underlie the performance of various membrane-associated and trans-membrane processes [18]. The obtained results showed a marked elevation of the unsaturated fatty acids and a decrease of the saturated ones. This is an interesting finding because the elevated level of unsaturated fatty acids is a prerequisite for higher oxidative attack because the unsaturated acyl chains are a perfect target for oxidative damage. The results presented in Fig. 2 support this notion, showing a statistically significant elevation of the lipid peroxides. Thus, PLA2, which participates in the de-acylation/re-acylation cycle, the latter being responsible for maintenance or remodelling of the phospholipid fatty acid composition, occurs as an important factor, supplying the membranes with acyl chain substrate, which is highly susceptible to oxidation.

In conclusion, we suggest that quercetin induces elevation of the lipid peroxides and oxidative stress in general in human adenocarcinoma cells through the activation of PLA2, which is responsible for augmentation of the level of susceptible to oxidative attack acyl chains. These findings shed light on the mechanism by which the flavonoid quercetin enhances the oxidative stress in certain tumour cells.

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