

Effects of Quercetin and Vitamin D on MCF-7 Cell Proliferation with Real-Time Cell Analysis

Gulsum Tekin¹, Ali Unlu², Bahadir Ozturk³

^{1,2,3}Selcuk University Faculty of Medicine, Department of Biochemistry

Correspondence to: Gülsüm Tekin, Selcuk University Faculty of Medicine, Department of Medical Biochemistry, Alaeddin Keykubad Campus, 42075 Selcuklu/Konya, Turkey

Abstract: The aim of this study was to investigate the effects of Quercetin (Que) and vitamin D (vitD) alone and Que in combination with vitD on proliferation of human breast cancer cells (MCF-7) by real-time and continuous monitoring of cell proliferation, and viability. After seeding 200 μ L (10.000 cells/well) of the cell suspensions into the wells of E-plate 16, cells were treated with Que, vitD and Que+vitD mixtures and monitored every 15 min for 140 hours. Almost a day after seeding, the cells were treated with Que (100-125-150 μ M) and 140 nM vitD and Que+vitD mixtures. Antiproliferative effect of Que+vitD mixtures started to increase at ~45. hours compared to Que doses and at ~65. hours compared to vitD after treatment. Cell proliferation decreased to 71,4 % with 150 μ M mix, 61,7 % with 125 μ M mix and 57,4 % with 100 μ M mix for 80 hours compared to the control ($p=0,000$). When MCF-7 cells were treated with Que, vitD and Que+vitD mixtures, cell viability decreased significantly in time-dependent manner. Exposure of MCF-7 cells to Que+vitD mixture resulted in a clear decrease in viable cells and may be a new and alternative therapeutic approach to breast cancer.

Keywords: MCF-7; Proliferation; Vitamin D; Quercetin

1. Introduction

Flavonoids, are group of polyphenolic compounds, known to have significant anti-tumor, antioxidant and anti-inflammatory activities¹. Several dietary polyphenols, present in vegetables, fruits and beverages, are known to possess health protective, cancer preventive and anti-cancer effects². Dietary polyphenols have been correlated with a reduced risk of developing cancer³. Quercetin (Que), one of the main flavonoids present in fruits and vegetables particularly rich in this compound, such as onions, apples, and strawberries⁴.

Vitamin D or vitamin D analogues might have potential as anticancer agents because their administration has antiproliferative effects, can activate apoptotic pathways and inhibit angiogenesis. In addition, Vitamin D potentiates the anticancer effects of many cytotoxic and antiproliferative anticancer agents⁵.

Worldwide it is estimated that more than one million women are diagnosed with breast cancer every year and more than 410,000 will die from this disease^{6,7}. As such, despite the considerable number of available therapeutic strategies against breast cancer⁸, this pathology continues to be a great public health burden worldwide.

Therefore, developing new strategies for prevention of breast cancer progression and overcoming drug resistance represents a major challenge. The purpose of this study was to investigate the effects of Que alone and in combination with vitamin D on proliferation in human breast cancer MCF-7 cells by real-time and continuous monitoring of cell proliferation, and viability.

2. Materials and Methods

Quercetin dihydrate (Calbiochem, 551600), vitamin D (cholecalciferol sigma, c9756) and Que+vitamin D mixture,

E-plate 16 (ACEA Biosciences, Inc. cat no: 05469813001) were used in this study.

Cell Culture

An immortalized human breast cancer cell line (MCF-7) provided by Dr. Abdulkemir Bedir (Medical Faculty of Ondokuz Mayıs University, Samsun, Turkey) was used. All cells used in this study were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS, v/v) and 1% penicillin (100 U/ml) and streptomycin (100 mg/ml) at 37 °C incubator with 5% CO₂ saturation. The cells were passaged with 0.25% trypsin and 0.1% ethylene diaminetetraacetic acid. After seeding 200 μ L (10.000 cells/well) of the cell suspensions into the wells of E-plate 16, cells were treated with Que, vitamin D and Que+vitamin D mixture and monitored every 15 min for 161 hours. ~1 day after seeding, the cells were treated with 100 μ M Que, 125 μ M Que, 150 μ M Que and Vitamin D (50 μ M) and Que+Vitamin D mixtures. Then the changes were observed in cell proliferation for 161 hours by xCELLigence device (xCELLigence, Roche Diagnostics GmbH, Penzberg, Germany).

Cell Proliferation and Viability Assay Using xCELLigence System

The xCELLigence system (xCELLigence, Roche Diagnostics GmbH, Penzberg, Germany) was used to evaluate cell survival according to the instructions of the supplier. The xCELLigence system consists of 4 main components: the impedance based real-time cell analyzer (RTCA), the RTCA plate station, the RTCA computer with integrated software, and three disposable E-plate 16. The RTCA plate station fits inside a standard tissue-culture incubator. The electronic impedance of the sensor electrodes was measured to allow monitoring and detection of physiologic changes in the cells on the electrodes. The voltage applied to the electrodes during the RTCA measurement was approximately 20 mV (root mean square). The impedance measured between the electrodes in each well depends on the electrode geometry, ion concentration in

Volume 6 Issue 3, March 2017

www.ijsr.net

Licensed Under Creative Commons Attribution CC BY

the well, and attachment of the cells to the electrodes. In the absence of cells, the ion environment both at the electrode-solution interface and bulk solution mainly determines electrode impedance. In the presence of cells, cells attached to the electrode sensor surfaces act as insulators and alter the local ion environment at the electrode-solution interface, leading to increased impedance.

Thus, the obtained values of electrode impedance increase as the number of cells growing on the plate increases. The data expressed in cell index units can be exported to the Excel program for any type of mathematical analysis.

Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) and Tukey-HSD multiple comparison tests ($p < 0.01$) for the proliferation experiments. All

calculations were performed using the RTCA integrated software of the xCELLigence system. The RTCA software performs a curve fitting of selected sigmoidal dose-response equations to the experimental data points. The data are presented as means \pm SD deviation of triplicate wells.

3. Results

The online growth of the control and experimental groups of the cells were shown at Figure. The mean and standard deviation of three wells were used to calculate the differences between control group and experimental groups (table 1). Control group baseline cell indexes were 1,08 for 72. hours and 2,82 for 161. hours. The cells were shown a good proliferation curve for the whole experiment time.

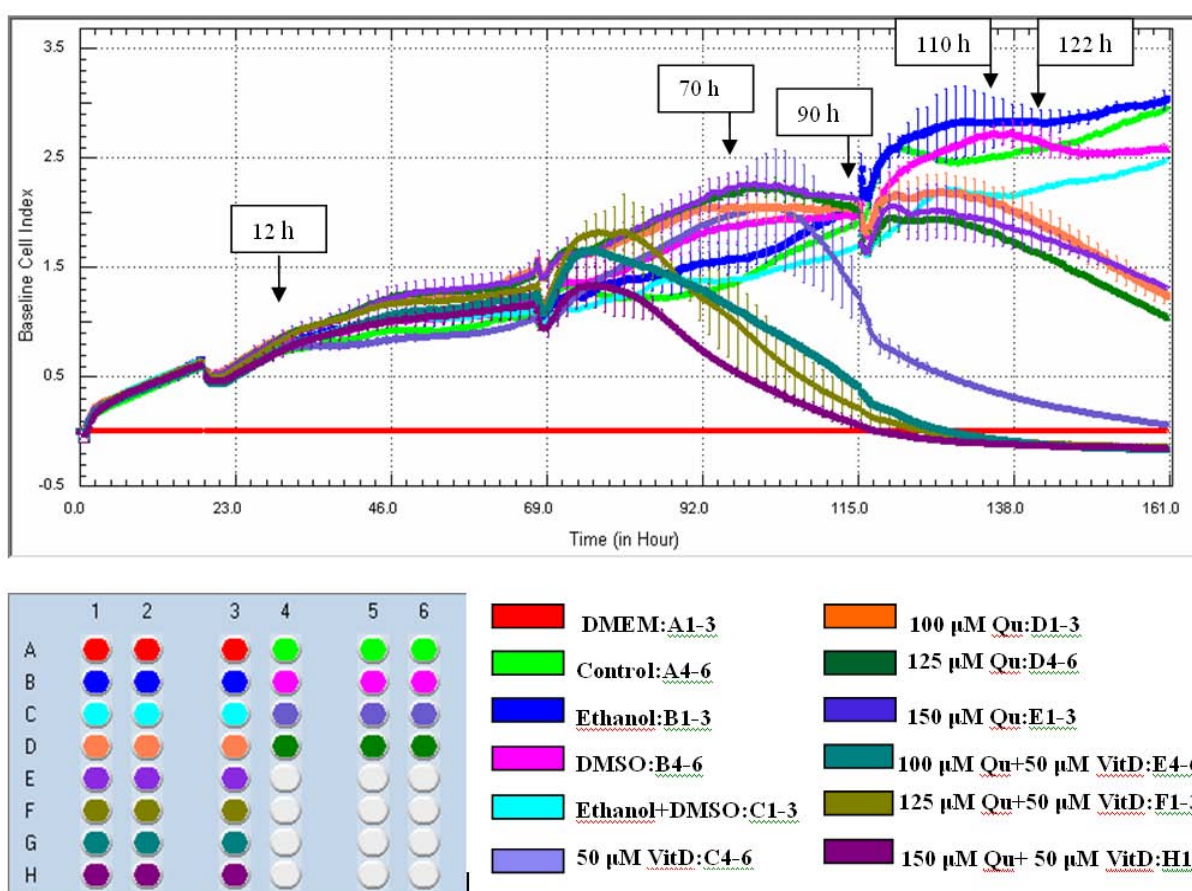


Figure: Real-time monitoring of cell adhesion and cell proliferation

Que alone did not inhibit the cell proliferation at the dose of 100, 125 and 150 μM during first 100 h of treatment (figure 1). Cell proliferation was decreased up to 16,3 % with 100 μM Qu, 23,8 % with 125 μM Qu and 24,6 % with 150 μM Qu at 110. hours compared to the control ($p=0,000$). Vitamin D related inhibition was not observed at initial 80 hours after treatment. Vit. D inhibited cell proliferation 84,5 % at 110 and 91,3% ($p=0,000$) at 122 h (table 2).

While vitamin D and Que still separately was uneffective, Que+vitamin D mixture started to inhibit cell proliferation at 50. hours after treatment. Inhibitor effect of 150 μM mix was observed earlier than other mix doses. Cell proliferation was decreased up to 91,9 % with 150 μM mix, 80 % with 125 μM mix and 75,1 % with 100 μM mix for 90 hours compared to the control ($p=0,000$). Cell proliferation was decreased 100 % with all mix doses for 110 hours compared to the control (table 2).

Table 1: Cell index as mean ± standard deviation (M±SD) on the MCF-7 cells at 12 h, 74 h, 110 h and 122 h after treatment

	12 h(M±SD)	74 h(M±SD)	110 h(M±SD)	122 h(M±SD)
CONTROL	0,83±0,021	1,254±0,08	c	2,52±0,13
DMSO	0,80±0,013	1,82±0,14	b	2,59±0,048
Ethanol	0,81±0,036	1,61±0,13	bc	2,86±0,13
Etanol+DMSO	0,81±0,016	1,33±0,14	c	1,77±0,052
50 µM VitD	0,74±0,042	1,89±0,14	b	0,39±0,035
100 µM Qu	0,85±0,019	2,09±0,07	ab	2,11±0,09
125 µM Qu	0,82±0,022	2,24±0,031	ab	1,92±0,13
150 µM Qu	0,83±0,025	2,28±0,081	a	1,90±0,08
100 µM Qu+50 µM VitD	0,81±0,019	1,07±0,14	c	0,00±0,001
125 µM Qu+50 µM VitD	0,79±0,026	1,18±0,11	c	0,00±0,021
150 µM Qu+50 µM VitD	0,79±0,021	0,60±0,061	d	0,00±0,012
P	0,23	0,00		0,00

a,b,c: Means with different letters in the same column are different

Table 2. Cell index as mean ± standard deviation (M±SD) and percentages of cell viability on the MCF-7 cells at 12 h, 90 h, 110 h and 122 h after treatment

	12 h	90 h	110 h	122 h
	Cell index(M±SD)	Cell via.(%)	Cell index (M±SD)	Cell via.(%)
CONTROL	0,83±0,021	100	1,85±0,09	100
50 nM VitD	0,74±0,042	89,2	1,30±0,06	70,3
100 µM Qu	0,85±0,019	102,4	2,1±0,05	113,5
125 µM Qu	0,82±0,022	98,8	2,1±0,09	113,5
150 µM Qu	0,83±0,025	100	2±0,05	108,1
100 µM Qu+ 50 nM VitD	0,81±0,019	97,5	0,46±0,08	24,9
125 µM Qu+ 50 nM VitD	0,79±0,026	95	0,37±0,09	20
150 µM Qu+ 50 nM VitD	0,79±0,021	95	0,15±0,05	8,1

4. Discussion

The most widely accepted physiological role of vitamin D, is in the physiological regulation of Ca²⁺ and Pi transport and bone mineralization⁹. However, recent observations indicate a much broader range of action for vitamin D, including the regulation of differentiation, proliferation and apoptosis¹⁰. Epidemiological observations have shown an association between low serum vitamin D levels and increased risk for breast cancer¹¹.

MCF-7 has a receptor for vit D¹². In our study exposure of MCF-7 cells to Vitamin D resulted in an approximate 29.7% at 90 h, 84.5 % at 110 h and 91.3% at 122 h decrease in viable cells after treatment. In literature plenty of study showed growth inhibition by vit D of the cancer cells.

Sundaram et al. have demonstrated that treatment vitamin D3 analog EB 1089 (100 nM) for 72 h reduced MCF-7 cells growth by ~50%. Combined treatment of cells with EB 1089, 72 h followed by adriamycin resulted in an approximately 90% reduction in final cell number compared to growth of untreated controls. The vitamin D3 analog EB 1089 enhances the antiproliferative effects of adriamycin six folds in MCF-7 breast tumor cells¹³.

Swami et al. demonstrates the effect of 1,25(OH)₂D₃ and its analogues on the growth of MCF-7 cells on the regulation of estrogen receptor (ER) in the estrogen-responsive MCF-7 human breast cancer cell line, which is known to predominantly express ERα and 1,25(OH)₂ D₃ caused a dose-dependent decrease in the cell growth. 1,25(OH)₂D₃

induced a dose-dependent decrease in ER levels, which was modest at 1 and 10 nM and higher (~50%) at 100 nM¹⁴.

Que shows specific inhibitory activity for cancer cell growth^{15,16}, but the mechanisms underlying the effects of Que in the induction of cell cycle arrest in human breast cancer cells are still unknown. Choi et al. examined the antiproliferative effect of Que on human breast cancer MDA-MB-453 cells at various concentrations (1-100 µM) and exposure times (3, 6, 12, and 24 hrs). Que significantly decreased the proliferation of MDA-MB-453 cells in time- and dose dependent manner. MDA-MB-453 cells treated with 100 µM Que for 24 hrs and resulted in a decreased number of G1 phase MDA-MB-453 cells (37.42% of the cell population compared with 60.81% for the controls)¹⁷.

Chou et al. were treated MCF-7 cells with Que for 24 and 48 h and at various doses (10-175 µM), cell viability decreased significantly in time- and dose-dependent manners. Que decreased the percentage of viable cells by approximately 12-90% in dose- and time-dependent manners, with an IC50 (50% inhibitory concentration) value of 92.4 µM after 48 h of treatment¹⁸.

Yang et al. exhibited significant antiproliferative activity of Quercetin 3-β-D-glucoside (Q3G) against MCF-7 cells at doses of 20-60 µM in a dose-dependent manner. The EC50 value of Q3G in inhibiting MCF-7 cell growth was 46.4 ± 1.3 µM¹⁹. Although above studies shown an inhibition of cell growth less than 125 uM of Que we did not observed any inhibition in online cell growth monitoring system for 100 hours. We had a significant inhibition over 100 uM doses after 100 h of treatment.

In the present work, we conducted experiments with a new real-time system that investigated the effects of the Que, vitamin D and Que+vitamin D mixture on MCF-7 cell line by real-time and continuous monitoring of cell proliferation and viability. Furthermore, the real-time cell analysis system allowed for calculation of time-dependent different concentrations, which can give more accurate information than single-value end points of classical proliferation and viability testing^{20,21}. Compared with conventional end point cell-based assays, real-time monitoring of cell response, such as cell adhesion, proliferation, and cell death, is an advantage of the real-time system to optimize the cell concentration for in-vitro assays; it also allows both cell and assay conditions to be constantly obtained before and during the experiments. Furthermore, the response of live cells can be monitored in real time; this is impossible with current end-point assays²².

As it can be seen from the data Que has a potential effect to increase vitamin D antiproliferative effects. Increased steroid receptor coactivators (NCoR1 and SMRT) have been found and implicated in the development of breast cancer cell lines. These coactivators repress the VDR-related activity. Que may have an effect on these coactivators. The potentiation effect of Que may be in the cell signalling pathway especially on EGFR related tyrosine kinase activity. It has been demonstrated that one of the induced apoptotic effect of vitamin D is the blockade of the MAPK signalling. Flavonoids have also a potent inhibitor of tyrosine kinase activity. Therefore it is possible to conclude that either vit D or Que induce each others antiproliferative activity by depressing the tyrosine kinase activity. It has been demonstrated that Que and resveratrol potentiates gefitinib (EGFR specific tyrosine kinase inhibitor) antiproliferative activity. This potentiation may be an increase in apoptosis. Increased caspase 3 activity by Que in MDA cells may have potentiate Vit D activity^{23,24}.

5. Conclusion

When MCF-7 cells were treated with 100-150 μ M Que, Vitamin D (50 μ M) and Que+Vitamin D mixture, cell viability decreased significantly in time-dependent manner. Exposure of MCF-7 cells to Que+Vitamin D mixture resulted in a clear decrease in viable cells. Combine vit D and flavonoid treatments may be a new and alternative therapeutic approach to breast cancer.

6. Conflict of Interest

The authors have declared that there is no conflict of interest.

7. Acknowledgements

This study was supported by Coordination Unit of Selçuk University Scientific Research Projects.

References

- [1] Hodek P, Trefil P, Stiborova M. Flavonoids-potent and versatile biologically active compounds interacting with cytochromes P450. *Chem Biol Interact* 2002; 139: 1-21.
- [2] Moreira L, Araújo I, Costa T, Correia-Branco A, Faria A, Martel F, Keating E. Que and epigallocatechin gallate inhibit glucose uptake and metabolism by breast cancer cells by an estrogen receptor-independent mechanism. *Experimental Cell Research* 2013; 319: 1784-1795.
- [3] Mandel S, Weinreb O, Amit T, Youdim MB: Cell signaling pathways in the neuroprotective actions of the green tea polyphenol (-)-epigallocatechin-3-gallate: implications for neurodegenerative diseases. *J Neurochem* 2004; 88: 1555-1569.
- [4] Russo M, Spagnuolo C, Tedesco I, Bilotto S, Russo GL. The flavonoid Que in disease prevention and therapy: facts and fancies. *Biochemical Pharmacology* 2012; 83: 6-15.
- [5] Deeb KK, Trump DL, Johnson CS. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. *Nature Reviews Cancer* 2007; 7: 684-700.
- [6] Jemal A, Bray F, Center M, Ferlay J, Ward E, Forman D. Global cancer statistics Ca-Cancer. *J Clin* 2011; 61: 69-90.
- [7] Parkin DM, Fernandez LMG. Use of statistics to assess the global burden of breast cancer. *Breast J* 2006; 12: 70-80.
- [8] Higgins MJ, Baselga J. Targeted therapies for breast cancer. *J Clin Invest* 2011; 121: 3797-3803.
- [9] Holick MF. Vitamin D and bone health. *J Nutr* 1996; 126: 1159-1164.
- [10] Kallay E, Pietschmann P, Toyokuni S, Bajna E, Hahn P, Mazzucco K, Bieglmayer C, Kato S, Cross HS. Characterization of a vitamin D receptor knockout mouse as a model of colorectal hyperproliferation and DNA damage. *Carcinogenesis* 2001; 22: 1429-1435.
- [11] Bertone-Johnson ER, Chen WY, Holick MF, Hollis BW, Colditz GA, Willett WC, Hankinson SE. Plasma 25-hydroxyvitamin D and 1, 25-dihydroxyvitamin D and risk of breast cancer. *Cancer Epidemiol Biomarkers Prev* 2005; 14: 1991-1997.
- [12] Diesing D, Cordes T, Fischer D, Diedrich K, Friedrich M. Vitamin D – Metabolism in the Human Breast Cancer Cell Line MCF-7. *Anticancer Research* 2006; 26: 2755-2760.
- [13] Sundaram S, Chaudhry M, Reardon D, Gupta M, Gewirtz DA. The vitamin D3 analog EB 1089 enhances the antiproliferative and apoptotic effects of adriamycin in MCF-7 breast tumor cells. *Breast Cancer Research and Treatment* 2000; 63(1): 1-10.
- [14] Swami S, Krishnan AV, Feldman D. 1 α ,25-Dihydroxyvitamin D3 Down-Regulates Estrogen Receptor Abundance and Suppresses Estrogen Actions in MCF-7 Human Breast Cancer Cells. *Clin Cancer Res* 2000; 6(8): 3371-3379.
- [15] Notoya M, Tsukamoto Y, Nishimura H, Woo JT, Nagai K, Lee IS, Hagiwara H. Quercetin, a flavonoid, inhibits the proliferation, differentiation, and mineralization of osteoblasts in vitro. *Eur J Pharmacol* 2004; 485: 89-96.
- [16] Ramos AA, Lima CF, Pereira ML, Fernandes-Ferreira M, Pereira-Wilson C. Antigenotoxic effects of Quercetin, rutin and ursolic acid on HepG2 cells: evaluation by the comet assay. *Toxicol Lett* 2008; 177: 66-73.
- [17] Choi EJ, Bae SM, Ahn WS. Antiproliferative effects of Quercetin through cell cycle arrest and apoptosis in

- human breast cancer MDA-MB-453 cells. *Archives of Pharmacol Research* 2008; 31: 1281-1285.
- [18] Chou CC, Yang JS, Lu HF, Ip SW, Lo C, Wu CC, Lin JP, Tang NY, Chung JG, Chou MJ, Teng YH, Chen DR. Quercetin-mediated cell cycle arrest and apoptosis involving activation of a caspase cascade through the mitochondrial pathway in human breast cancer MCF-7 cells. *Archives of Pharmacol Research* 2010; 33: 1181-1191.
- [19] Yang J, Liu RH. Synergistic Effect of Apple Extracts and Quercetin 3- β -D-Glucoside Combination on Antiproliferative Activity in MCF-7 Human Breast Cancer Cells in Vitro. *J. Agric. Food Chem.* 2009; 57: 8581-8586.
- [20] Urcan E, Haertel U, Styllou M, Hickel R, Scherthan H, Reichl FX. Real-time xCELLigence impedance analysis of the cytotoxicity of dental composite components on human gingival fibroblasts. *Dent Mater* 2010; 26: 51-58.
- [21] Ozturk F, Malkoc S, Ersoz M, Hakki SS, Bozkurt BS. Real-time cell analysis of the cytotoxicity of the components of orthodontic acrylic materials on gingival fibroblasts. *Am J Orthod Dentofacial Orthop* 2011; 140: 243-249.
- [22] Reichl FX, Esters M, Simon S, Seiss M, Kehe K, Kleinsasser N, Folwaczny M, Glas J, Hickel R. Cell death effects of resin-based dental material compounds and mercurials in human gingival fibroblasts. *Arch Toxicol* 2006; 80: 370-377.
- [23] Deeb KK, Trump DL, Johnson CS. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. *Nature Reviews Cancer* 2007; 7: 684-700.
- [24] Castillo-Pichardo L, Dharmawardhane SF. Grape polyphenols inhibit Akt/Mammalian target of rapamycin signaling and potentiate the effects of gefitinib in breast cancer. *Nutrition and Cancer* 2012; 64: 1058-1069.