Investigation of the Effect of Quercetin in MCF-7 Breast Cancer Cells

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Abstract

In this study, it was aimed to investigate the effects of Quercetin against in vitro cell proliferation of the human breast cancer cell line MCF-7. MCF-7 were treated by 25 μM/ml, 50 μM/ml and 100 μM/ml of Que for 48 hours MCF-7. Then, the viability rates in the cells were determined by the MTS test. The cells in the experimental group increased depending on the dose. Mean cell numbers were 51.9 × 10^3 (± 1.4), 32.5 × 10^3 (± 1.05), 24.3 × 10^3 (± 1.11) and 21.2 × 10^3 in the control and experimental groups, respectively. When the control group and the experimental groups were compared, it was found that the decrease in the number of cells in the experimental groups was statistically significant (p < 0.05). As a result of this study, the apoptotic effect of Quercetin was demonstrated in the MCF-7 cell line. Future studies are needed to elucidate cell death signaling pathways in cancer cells treated with Que.

Keywords: Quercetin; MCF-7; Angiogenesis

Introduction

Cancer chemopreventive agents induce apoptosis in various cancer cell lines by inhibiting cell growth and proliferation. It also provides a new therapeutic target against tumor spread by blocking angiogenesis [1,2].

Many chemotherapeutic drugs for anticancer activity have been investigated. The search for new compounds for cancer treatment forms the basis of a large number of studies and many researchers focus on plant-derived compounds with healing potential. Quercetin (Que) is one of the most abundant naturally occurring flavonoid and it has been found to be very efficacious against many kinds of cancer cells [3,4]. Que has a strong anti-proliferative effect against tumor cells and by stabilizing microtubules, blocking mitosis and inducing apoptosis.

Aim of the Study

The aim of this study is to investigate the effects of different doses of Que against in vitro cell proliferation of the human breast cancer cell line MCF-7.

Material and Method
Design cell culture

In this study, MCF-7 cells (SCC101, Sigma-Aldrich) seeded from a commercially produced cell line were used. MCF-7 in medium containing streptomycin/penicillin (100 U/mL; Sigma, St. Louis), 10% fetal bovine serum (Sigma, St.Louis) in 5% CO₂ humid incubator at 37°C and RPMI 1640 was cultured. Healthy MCF-7 cells were then grouped for Que administration.

Experiment groups

Cells were counted on the thoma slide and then divided into 4 groups, 1 control and 3 experimental. Then 1 x 10⁴ cells were seeded into each well. After the cells seeded in 96-well plates were kept in the incubator overnight, the wells were washed twice with PBS and the medium was changed. After 48 hours of incubation, only medium was added to the control group, while medium and 25 μM/ml, 50 μM/ml and 100 μM/ml Que were added to the experimental groups. To count MCF-7 cells, the cells in the culture dish were then washed three times with PBS and 20 μL trypsin/EDTA was added and kept at 37°C for 1-2 minutes. Then 40 μL of medium was added and centrifuged twice for 10 minutes at 1000 rpm. After adding 20 μL of medium to the pellet remaining at the bottom of the eppendorf, pipetting was performed. 10 μL of the suspended mixture was taken and transferred to another eppendorf tube. Pipetting was performed by adding 10 μL of trypan blue. 10 μL of the prepared suspension was transferred to the Thoma slide and the cells were counted by adjusting to x40 under the microscope.

MTS analysis

The viability of cells were detected by MTS assay (Promega, Madison, WI, USA), after cells treatment, to measure cell growth. Cells were stained with trypan blue and counted using a hemocytometer and viable cells were detected. Viable cells were inoculated into 96-well plates (1 x 10⁴ cells/well). Then 25 μM/ml, 50 μM/ml and 100 μM/ml Que were added. After 48 hours of treatment, MTS (3- (4,5-dimethylthiazol-2-yl) -5- (3-carboxy-methoxypenyl) -2- (4-sulphophenyl) -2H-tetrazolium) and PMS (Phenazine methosulfate to cells) (20: 1 v/v) was added. After 2 - 3 hours incubation at 37°C, viable growing cells were predicted by monitoring the absorption of the product at 490 nm, based on formazan formation by living cells. All groups were repeated three times and results reported as mean absorption ± standard deviation.

Statistical analysis

The obtained data were expressed as “mean value ± standard error” (x ± SEM). Significance between groups was determined using the one-way ANOVA test followed by Fisher’s Post-Hoc. All statistical procedures were performed according to the “IBM SPSS Statistics Version 20” statistical program and p < 0.01 was considered significant.

Results

It was observed that the cells counted in the hemacytometer were significantly decreased in the experimental groups due to the increasing dose of Que (Table 1). The average number of cells in the control and experimental groups was 51.9 × 10³ (± 1.4), 32.5 × 10³ (± 1.05), 24.3 × 10³ (± 1.11) and 21.2 × 10³ (± 0.89), respectively. When the control group and the experimental groups were compared, it was found that the decrease in the number of cells in the experimental groups was statistically significant (p < 0.05).

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Mean (± standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16</td>
<td>51.9 × 10³ ± 1.4</td>
</tr>
<tr>
<td>25 μM/ml Que</td>
<td>16</td>
<td>32.5 × 10³ ± 1.05</td>
</tr>
<tr>
<td>50 μM/ml Que</td>
<td>16</td>
<td>24.3 × 10³ ± 1.11</td>
</tr>
<tr>
<td>100 μM/ml Que</td>
<td>16</td>
<td>21.2 × 10³ ± 0.89</td>
</tr>
</tbody>
</table>

Table 1: The effect of Que on in vitro MCF-7 cell proliferation. Data were expressed as mean ± standard deviation.
Experimental groups (25 μM/ml, 50 μM/ml and 100 μM/ml Que) were found to be statistically significant compared to each other, showing a decreased dose of cells due to high dose (p < 0.05) (Figure 1). In addition, the results of the regression analysis showed that cell viability decreased depending on the dose, that is, cell death increased as the dose increased.

![Figure 1: Cell distribution between groups.](image)

**Discussion**

Angiogenesis, which means the formation of new capillaries from preexisting blood vessels, is a fundamental process involved in various physiological and pathological processes [5]. Induction of blood flow causes growth and metastasis of solid tumors [6]. In fact, angiogenesis has become a promising process for experimental treatments in cancer, and it has been promising in the development of various treatments [7].

Uncontrolled growth of cells is defined as cancer. Breast cancer is among the most frequently diagnosed and leading cause of cancer deaths among women worldwide. Chemotherapy and radiation treatments are insufficient due to adverse effects on healthy cells and drug resistance [8]. In recent years, it has been reported that it has increased the survival rate after diagnosis, depending on the improvements in diagnosis and treatment. In general, methods such as surgery, radiotherapy, chemotherapy and immunotherapy are used in cancer treatment [9,10]. Cancer is one of the most fatal diseases and therefore research in this area has increased. While some of these studies focus on cancer biology, some are done to improve the ways of treatment. Cell lines obtained from tumors developed in vitro, in vivo or spontaneously can also be used in studies on cancer. One of them is MCF-7. The MCF-7 cell line, which is the most commonly used in laboratory research around the world, is a weak proliferative and non-invasive cell line with generally low metastatic potential [11]. MCF-7 cells are universally used in research for ER (estrogen receptor) positive breast cancer cells. MCF7 cells have been reported to be compatible with anti-hormone therapy resistance studies because they are easily cultured and have ER expression when treated.

MCF-7 cell populations modified in various anti-hormone media have been generated to investigate the properties of anti-hormone-resistant breast cancer cells. MCF-7 cell line provides practical information about breast cancer diseases [12].
Quercetin is a flavonoid commonly found in nature. Quercetin has been classified as an antioxidant in vitro by inhibiting the oxidation of other molecules. It contains a polyphenolic chemical infrastructure that stops in vitro oxidation by acting as a scavenger of free radicals. Quercetin has been noted to have potential effects in preventing diseases, including cancer.

It has also been reported that in human breast cancer cell lines, quercetin acts as an agonist of the estrogen receptor bound to the G protein [13].

In our study, it has been shown that quercetin has antiproliferative effects at 100 µM. This is compatible with other studies. As an example, 100 µM quercetin in human PC-3 prostatic cells [14], 100 µM quercetin in human tongue squamous carcinoma cells [15], 10 to 70 µM quercetin in human stomach cell lines [16], 15 - 120 µM quercetin in HT-29 and Caco-2 cells [17] and HT29 [18] have been shown.

**Conclusion**

In our research, the effect of Que on MCF-7 has been evaluated. Apoptotic and viable cells were counted by the MTS test method. The results of this investigation demonstrated apoptotic events, a significant reduction in cell viability in the Que-administered MCF-7 cell line. These results are consistent with other in vitro and in vivo studies showing that treatment of cancer cells with Que causes apoptosis induction. New studies are needed to increase our knowledge of cell death signaling pathways in cancer cells treated with Que.

**Bibliography**


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