Assessment of Anticancer Potential of Quercetin against Breast, Colon and Colorectal Cancer Cell Lines and Related Cell Cycle and Apoptotic Gene Profile: In Vitro Study

Habiba Shedid¹, Eman Amin Ismail² & Aly Fahmy Mohamed²

Abstract: Compounds naturally derived had shown a great effect in the treatment and the prevention of cancer. Quercetin is naturally derived compound found in fruits and vegetables. In the current study, we tested the effect of Quercetin on breast (MCF7 and MDA-MB-231), colon (Caco2) and colorectal (HCT-116) cancer cell lines. We found that the IC50 values were cell type dependent as breast cancer cell line was mostly affected than colon and colorectal cell lines. Quercetin IC50 treated cells showed a variable reactivity and were arrested at the G2-M phase in colon, colorectal and breast cancer cell lines. Therefore, our data propose that Quercetin can be used in cancer therapy.

Key words: Quercetin, Breast cancer, colon and colorectal, cell cycle, apoptosis

1. Introduction

Cancer cells differ from normal cells proliferate anywhere in the body. It may be a genetic disease which can be inherited and arises due to a genetic disorder that affect the cell proliferation (Ranganathan, Halagowder, & Sivasithambaram, 2015) metastatic cancer that spread from its original site to another (Lugassy, & Escande, 1997). Symptoms of cancer are type and location dependent. One of the most frequent worldwide type of cancer and especially in the Western countries is the colon cancer. Depending on the diet and specifically for the Western’s, studies are made to indicate that the high consumption of red meat in one of the causes of colon cancer (Boateng et al., 2007). However, another type of cancer; the mostly diagnosis type of cancer nowadays is the breast cancer (“Fact Sheets by Population”, 2017). Its progression comes in multistep which contain hormones, genes and recently discovered is the developmental genes (Yang et al., 2004). As cancer cells proliferate uncontrollably, cells use the body energy and hormones which may lead to the cause of fever, fatigue, anemia and an unexplained weight loss. Traditional treatments that are mainly used are: surgery, radiotherapy and chemotherapy. These traditional ways of treatment are used to eliminate the danger of cancer from spreading or recurrence, not withstanding all these effort done to cure cancer, treatments can cause many side effects like mouth infection, hair loss, bleeding, vomiting and others. Therefore, alternative treatments are done naturally or artificially with less side effects and similar efficacy, A new trend for the use of natural derived Quercetin that could be detected in many foods and plants like red wine, onions, green tea, apples and others (Erlund, 2004). Quercetin is used for treating many conditions like high cholesterol, heart disease, cancer and others, treatment studies have also shown their ability to inhibit tumour growth. It is used due to the presence of antioxidants, those can induce cell membrane damage, interact with the DNA and cause cell death (“Quercetin - Penn State Hershey Medical Center”, 2017).

The current study aimed to evaluate the anticancer potential against breast (MCF-7 and MDA-MB-231) and colon (CACO-2 and HCT 116) cancer cell lines and related cell cycle and genetic profile.

1.1. Materials and methodology

Quercetin was kindly supplied from (Sigma – Aldrich – USA), it was prepared in Dimethyl sulfoxide (DMSO) as 10 mM starting concentration.

1.2. Cell culture

Breast (MCF-7 and MDA-MB-231), colon and colorectal cancer cell lines (Caco2 and HCT-116) were kindly supplied from VACSERA, tissue culture department in a complete growth medium; DMEM, 10% and 1% antibiotic (GIBCO-USA). Cells were maintained according to manufacture protocol, where exhausted growth media were decanted under sterile condition using vertical air laminar air flow (NUAir-USA), cells were treated with 0.25% trypsin solution (Sigma, Aldrich – USA) for 5-10 minutes at 37°C,
detached cells were resuspended in growth media and cell count was adjusted as $2 \times 10^5$/ ml. Cells were dispensed in both cell culture plates and TC flasks (TPP-Swiss) according to the applications.

1.3. Cell viability assay

Cell viability was performed using MTT assay, as cells were cultured in a 96 well plate as $5 \times 10^2$ cells/ml. One day after cell seeding, the medium was changed and the Quercetin prepared as 10 mM was 2 fold serially diluted in maintenance medium and dispensed to the plates 0.1 ml/well. Plates were incubated for 24 h (Jouan-France). De-attached cells were washed out using (PBS) phosphate buffer saline (ADWIA EGYPT). Residual live cells were stained with MTT (0.05%) for 4 hours. Developed MTT formazan crystals were dissolved using DMSO as 50µl. Developed colour intensity was measured using ELISA plate reader (ELX-800-Biotek-USA) at 570 nm absorbance was plotted against Quercetin concentrations. IC$_{50}$ value was calculated using Master plex 2010 software.

1.4. Flow cytometry analysis

$2 \times 10^5$ cells of test cancer cell lines were plated in 25 Cm$^2$ surface area tissue culture flasks. And 24 h later the growth media were decanted and cells were treated with the IC$_{50}$ value of Quercetin prepared in RPMI-1640. Treated flasks were examined for detection of cytopathic effect. De-attached and adherent cells were harvested, cold centrifuged (Jouan-Ki-22) for 15 minutes at 2000 rpm and pelleted cells were dispensed in 1ml PBS and divided in different eppendorph tubes for performing molecular and cell cycle analysis.

1.5. Polymerase chain reaction (PCR)

Assessing the expression of P53, Bcl-2 and Bax by the usage of cDNA template for PCR. 25 μl dream Taq green master mix, 4 μl cDNA, 2 μl each of forward and reverse primer (10 picomole/μl) and at 94 °C for 3 minutes 17 μl nuclease free water were pre-denaturated. Amplification was performed (35 cycles) with each cycle consisting of denaturation at 94°C for 30 secs, annealing at 57°C (p53, bax) and (Bcl-2) for 30 sec followed by extension at 72°C for 45 sec. Heating at 72°C for 5 min and then the reaction is terminated. To confirm the absence of genomic DNA non-reverse transcribed RNAs were included. to assess for reagent contamination, negative control without adding template was also included Huang et al., (2006). Housekeeping gene (β-actin was included).

1.6. Statistical analysis

All experiments were carried out in three independent tests. Data were expressed as the mean standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA). The results were considered statistically significant at probability <0.05.

2. Results

2.1. Cytotoxicity

The viability of Quercetin treated cells were monitored using MTT assay and data recorded revealed that the cell viability was concentration and cell type dependent recording an IC$_{50}$ value in the order of 942, 1353, 1442 and 1274 for Caco-2 and HCT-116 [Fig 1 - 3] and MDA-MB-231 and MCF-7 [Fig 2- 4] respectively. It was noticed that Caco-2 was mostly affected than HCT-116 in the same ay MCF-7 was affected insignificantly than MDA-MB-231.

2.2. Cytotoxicity

[Fig.2] Cell viability of Caco-2 and HCT treated with Quercetin.

Primers

<table>
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<th>Primers</th>
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<tr>
<td>P53 F 5’-CCCCTCGTGCCCTGTCATCTTTC-3’</td>
<td>F 5’-CCCCTCGTGCCCTGTCATCTTTC-3’</td>
<td>P53 R 5’-GCAGCGCCTCACAACCTCCGTAC-3’</td>
<td>R 5’-GCAGCGCCTCACAACCTCCGTAC-3’</td>
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<tr>
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<td>F 5’-GTGTCTCCAAGGAGATGGCTGAT-3’</td>
<td>Bax R 5’-CATCTTTCTTGGATGGTCTAAGGAC-3’</td>
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</tr>
<tr>
<td>Bcl-2 F 5’-CTTCTGTAGACGATGGTCTAAGGAC-3’</td>
<td>F 5’-CTTCTGTAGACGATGGTCTAAGGAC-3’</td>
<td>Bcl-2 R 5’-GAGAGACTTCCACACCCACAGGAC-3’</td>
<td>R 5’-GAGAGACTTCCACACCCACAGGAC-3’</td>
</tr>
<tr>
<td>β-actin F 5’-GTGACATCCACACCCAGAGG-3’</td>
<td>F 5’-GTGACATCCACACCCAGAGG-3’</td>
<td>β-actin R 5’-ACAGGATGTCAAAACTGCCC-3’</td>
<td>R 5’-ACAGGATGTCAAAACTGCCC-3’</td>
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[Fig.1] Primers sequences.
2.2. Cell cycle

Anticancer potential of Quercetin as natural derivative of different natural products was clear as the effect of Quercetin on the cell cycle profile indicating that the arresting was cell line dependent where the arresting phase of G0-G1 was significantly elevated in MCF7 than in MDA-MB-231 (P<0.05) while the opposite was noticed where the G2-M phase arrest was significantly elevated in MDA-MB-231 cell line than MCF7 (P<0.05) [Fig 1]. In the mean times the arrest profile in colon and colorectal cell lines showed that the predominant arrest phase was G2-M phase in Caco-2 cell line was significantly elevated than in HCT-116 (P<0.05) [Fig 2].

Molecular biology

Regarding the pro-apoptotic genes P53 and Bax there was a clear significant elevated increased level of genes expression in a significant way compared with cell control (P<0.05) and the expression fold increase was cell type dependent and MDA-MB-231 was the most affected by Quercetin treatment, followed by MCF-7, HCT-116 and Caco-2 respectively [Fig 4]. In the same time there was a significant down regulation of anti-apoptotic gene (BCL-2) post cell treatment. Also, MCF-7 was the most affected cells followed by Caco-2, MDAMB-2331 and HCT-116.
3. Discussion

Cancer is known to its ability of cells to proliferate uncontrollably with potential to invade and spread all over the body. Therefore, treatments for that type of disease should have been powerful and fast to cure the body. Moreover, the effectiveness of this treatment is clear but it has many side effects. Treatments regimen depends on the type of cancer and the stage but all are common in their side effects including hair loss, tiredness and others. Hence, to get over the side effects of these traditional treatments. Naturally derived products was discovered for dealing with cancer namely Quercetin. It is one of the richest compounds found in fruits and vegetables which has an anticancer and an anti-inflammatory effect (Mertens-Talcott & Percival, 2005). The effect of Quercetin as anticancer is due to different cell signalling mechanisms and its capability to inhibit enzymes and responsible for the activation of carcinogens and that by binding to the receptors and the enzymes of the cancer cell (Canivenc-Lavier et al., 1996). Based on researches done before, the safe dose of the Quercetin had the ability to down regulate the P53 protein responsible for breast cancer. The inhibition of gene expression was found to arrest the cells in the G2-M phase of the cell cycle, while in human Leukaemia T-cells cells were arrested in the late G1 phase of the cell cycle. Another study has reported that the intravenous administration of Quercetin had an effect on tyrosine kinase which involved in the transduction of growth factor signals to the nucleus. Another mechanism for the progression of cancer is inhibiting by Quercetin which is the heat shock protein in several cell lines like breast cancer (Hansen, Oesterreich, Lemieux, Sarge & Fuqua, 1997), Leukaemia (ELIA & SANTORO, 1994) and colon cancer (Koishi et al., 1992). The heat shock protein let the cancer cells to bypass the normal mechanism of the cell cycle arrest and also allow these cells to adapt to the body stress (Ciocca et al., 1993). Previous reports showed that Quercetin induced cytotoxicity in various human cancer cell lines, with varying sensitivity (Mertens-Talcott & Percival, 2005). Quercetin induced cytotoxicity in leukemic cells effectively (CEM, K562 and Nalm6), Nalm6 being most sensitive with an IC50 value of 20 μM which is different from our current study that may be attributed to the cell type, start concentration and treatment durations. Regarding the anti-proliferative potentials of Quercetin, our recorded toxicity of Quercetin was matching the (Mertens and Percival 2005) despite the usage of combined Quercetin with ellagic acid that show great effects in treating cancer cell lines. For ellagic acid IC50 was 170, >250 and 183 μM for Nalm6, K562 and CEM, respectively which indicates a higher toxicity of Quercetin in all cancer cells. Since Quercetin cytotoxicity is much higher than the ellagic acid the effect was assessed in human breast cancer cell lines, T47D using MTT assay. Results shown a reduced sensitivity to Quercetin with an IC50 of 160 μM while when treated on mouse breast cancer cell line, EAC, it showed much higher sensitivity IC50 of 50 μM but no effect when ellagic acid is used (Srivastava et al., 2016).

4. Conclusion

In vitro studies presented here indicated the ability of Quercetin as naturally extracted product could be used against cancer cell lines. Anticancer potential was concentration and cell type dependent accompanied with cell arrest mainly during the G2-M phase and the G0-G1 phase according to cell type, and the gene expression profile was cell type and concentration dependent as well.

5. Recommendation

Reformulation of Quercetin in a way to facilitate its oral and subcutaneous administration regarding the biochemical reactions related to anticancer potential.

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Twist, a Master Regulator of Morphogenesis, Plays an Essential Role in Tumor Metastasis. Cell, 117(7), 927-939.
