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RESEARCH ARTICLE

Effect of quercetin on secretion and gene expression of leptin in breast cancer

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Abstract

OBJECTIVE: To investigate the possible inhibitory action of pure quercetin on secretion and gene expression of leptin in the T47D breast cancer cell line.

METHODS: In this experimental study, T47D cells were cultured in monolayers in RPMI 1640. IC_{50} was determined by MTT (3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) assay after 24 h treatment at different concentrations of quercetin. The levels of leptin gene expression were measured by reverse-transcription real-time polymerase chain reaction (PCR). Secreted leptin was measured in the supernatant of cells by an enzyme-linked immuno sorbent assay.

RESULTS: Analysis of MTT assay data showed that quercetin has a cytotoxic effect on T47D breast cancer cells with 40 μM IC_{50} after 24 h exposure. Data analysis of real-time PCR showed that with increases in quercetin concentration, a decreasing trend was seen in mRNA levels of leptin of treated cells compared with the control cells ($P < 0.05$). Also, measurement of secreted leptin in the culture media showed a similar decreasing trend in the amount of leptin protein in the treated cells compared with the control cells ($P < 0.05$).

CONCLUSION: Quercetin significantly inhibits the growth of T47D cells through inhibition of leptin secretion and gene expression in T47D breast cancer cells. Therefore, it might be an alternative approach to breast cancer therapy through leptin targeting.

Keywords: Quercetin; Cytotoxicity; Real-time polymerase chain reaction; Breast neoplasms

INTRODUCTION

Breast cancer is one of the most commonly diagnosed cancers worldwide. The incidence of breast cancer is approximately 10.4%, and it is the most common cancer in women, showing an annual 1%-2% increase.[1,2] Because of the high morbidity and mortality of breast cancer, the development of an effective drug for its treatment is obviously required.

There are many known risk factors for breast cancer including age, sex, hormonal factors, diet, radiation exposure, breast density, family history, obesity, and mutations in susceptibility genes (BRCA1, BRCA2, TP53, PTEN and leptin). Leptin is expressed in normal mammary tissue, breast cancer cell lines, and solid tumors.[1-3] Leptin acts as a mitogen and induces growth and transformation of T47D breast cancer cells.[4] Also, it has been shown that increased levels of leptin and de-

creased adiponectin secretion are directly associated with breast cancer development.[2] With increasing attention paid to the significant role of leptin as a therapeutic target in breast cancer, natural compounds capable of reducing the expression and secretion of leptin could contribute greatly to preventing and treating breast cancer. Medicinal plants and their compounds are rich sources for discovering drug therapies and they can be used to prevent and treat diseases.[5-13]

Quercetin is a plant flavonoid that is generally found in citrus fruits, onions, tea, and red wine. It exhibits a wide range of properties such as anticarcinogenic, anti-inflammatory, and anti-viral actions. Many studies have shown that quercetin is able to induce cytotoxic effects, including inhibition of cell proliferation and apoptosis in a variety of cancer cells.[14-17]

In this investigation, we aimed to study the effect of quercetin on secretion of and leptin gene expression in the epithelial-like cell line T47D.

MATERIALS AND METHODS

Chemicals and reagents

Quercetin (2-[3,4-dihydroxyphenyl]-3,5,7-trihydroxy-4H-chromen-4-one, "Sigma-Aldrich provides this product to early discovery researchers as part of a collection of unique chemicals"), MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), glycine, and NaCl were obtained from Sigma (Deisenhofen, Germany). The leptin ELISA kit was purchased from Labor Diagnostika Nord GmbH and Co., KG (Nordhorn, Germany); fetal bovine serum (FBS) and phenol-red free Roswell Park Memorial Institute (RPMI) 1640 with L-glutamine were purchased from Gibco BRL (Life Technologies, Grand Island, NY, USA); and the T47D cell line was obtained from the National Cell Bank of Iran (Tehran, Iran). Sodium bicarbonate, streptomycin, and amphotericin B were purchased from Merck (Darmstadt, Germany); penicillin G was purchased from SERVA Co., (Heidelberg, Germany); Total RNA Isolation (TRIzol) reagent was purchased from Invitrogen (Eugene, OR, USA); the First-Strand cDNA Synthesis kit was purchased from Fermentas (Hanover, MD, USA); and the Syber Green-I reagent was purchased from Takara Bio (Otsu, Japan).

Cell culture

The T47D cell line was provided by the National Cell Bank of Iran (Tehran, Iran). The T47D cells were cultured in RPMI 1640 (+ L-glutamine) supplemented with 10% FBS, streptomycin (100 mg/L), penicillin G (100 IU/mL), and amphotericin B (2.5 μg/mL) and incubated at 37 ℃ in a humid atmosphere containing 5% CO_2.

Cell treatment and MTT assay

The cytotoxic effect of quercetin on T47D cells was investigated by MTT assay after 24 h of treatment. In short, 2000 cells/well were cultivated in a 96-well culture plate. After 24 h incubation at 37 ℃, cells were treated with different concentrations of quercetin (0-120 μM). Then, the medium of all wells was removed carefully and 50 μL MTT (2 mg/mL) was added to each well and incubated for 4.5 h in the dark, followed by the addition of 200 μL DMSO. Thereafter, Sorensen's glycine buffer (0.1 M glycine, 0.1 M NaCl, pH 10.5) was added and the absorbance of each well was read at 570 nm within 15-30 min. For data analysis, the mean OD of each well was calculated.

Total RNA extraction and cDNA synthesis

Total RNA was extracted by the TRIZOL Reagent according to the manufacturer's instructions. The concentration of prepared RNA was measured by a NanoDrop spectrophotometer (Termoscientific, Wilmington, DE, USA) and its integrity was confirmed by electrophoresis on a 1.2% agarose gel containing 1% formaldehyde.

After the RNA was prepared, cDNA was synthesized by the First-Strand Synthesis kit according to the manufacturer's instructions. The synthesized cDNA was immediately used in real time PCR or stored at − 70 ℃ for later use.

Real-time PCR

The real-time PCR method was used for analysis of leptin gene expression levels in the control and treated T47D cells. The real-time PCR reaction was done in triplicate using the Syber Green Ⅰ reagent in the Rotor Gene™ 6000 system (Corbett Research, Sydney, Australia) according to the manufacturer's instructions. The amplification conditions were as follows: leptin (2 min at 95 ℃ and a two-step cycle of 95 ℃ for 15 s and 60 ℃ for 40 s for 40 cycles). Changes in leptin gene expression levels between the control and treated T47D cells were calculated by the $2^{-\Delta\Delta CT}$ method.[18]

Measurement of the secreted leptin

To measure the possible effect of quercetin on the amount of secreted leptin in the treated cells compared with the control cells, leptin concentrations were measured in the supernatant from the cells by a human leptin ELISA kit (Labor Diagnostika Nord GmbH and Co., KG; Nordhorn, Germany) according to the manufacturer's instructions.

Statistical analysis

Data were expressed as mean ± standard deviation ($\bar{x}$ ± s). Data were processed with SPSS 18.0 (SPSS Inc., Released 2009. SPSS Statistics for Windows, version 18.0. Chicago, IL, USA). The differences in expression levels of leptin between the control and treated cells were analyzed by one-way analysis of variance and Dunett's multiple comparison tests. $P < 0.05$ was defined as significant.