RESEARCH ARTICLE

Effects of Genistein and Synergistic Action in Combination with Tamoxifen on the HepG2 Human Hepatocellular Carcinoma Cell Line

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Abstract

Introduction: The flavonoids comprise a diverse group of polyphenolic compounds with antioxidant activity that is present in edible plants like soybeans and soy products. In vivo studies have concentrated on the effects of flavonoids on cancer and genistein (GE), a soy-derived isoflavone, has been reported to reduce prostate, colon, hepatic and breast adenocarcinoma risk. Tamoxifen (TAM) is an important drug for cancer treatment worldwide, which can induce apoptosis in various cancers, including examples in the liver, breast and ovaries. The aim of the present study was to evaluate the effects of GE and TAM, alone and in combination, on proliferation and apoptosis in the human hepatocellular carcinoma (HCC) HepG2 cell line. Materials and Methods: HepG 2 cells were treated with GE, TAM and GE/TAM and then MTT and flow cytometry assays were conducted to determine effects on viability and apoptosis, respectively. Results: GE and TAM inhibited cell proliferation and induced apoptosis in the HepG 2 cell lines. Discussion: Our findings clearly indicated that GE and TAM may exert inhibitory and apoptotic effects in liver cancer cells. Conclusion: GE and TAM can significantly inhibit growth of HCC cells and play a significant role in apoptosis.

Keywords: Genistein- Tamoxifen- proliferation- apoptosis- hepatocellular carcinoma

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Introduction

The flavonoids, natural antioxidants, comprise a diverse group of polyphenolic compounds with antioxidant activity, including more than 4,000 phenylbenzopyrones that present in many edible plants (Nagendran et al., 2006; Harborne et al., 2000). This group contains distinct classes such as flavans and proanthocyanidins, flavonols, anthocyanidins, flavones, flavanones, neoflavonoids and isoflavones. These natural antioxidant compounds present in the legumes, cereals, fruits, nuts, seeds, vegetables, spices, herbs, stems and flowers, tea and cocoa (Harborne et al., 2000; Tim, 2005; Kutaiba et al., 2012; Crozier et al., 2009; Wang et al., 2009; Pier-Giorgio, 2000). In vivo studies have concentrated on the effects of flavonoids on cancer and have reported a variety of actions, including cell cycle arrest, carcinogen inactivation, induction of apoptosis and differentiation, antiproliferation and inhibition of angiogenesis (Chithan et al., 2005). Similar in vivo works have demonstrated that flavonoids inhibit tumor cell growth and induce cell differentiation (Middleton et al., 2005; Haitao et al., 2008; Robert et al., 2001; Monasterio et al., 2004; Hyon et al., 2005; Koen et al., 2005; Wen-Xin et al., 2005). Epidemiological studies have demonstrated that high dietary intake of flavonoids can decrease colon cancer prevalence in humans (Evropi et al., 2007; Uwe et al., 2000). Experimental investigations have showed that flavonoids induce cell cycle arrest in colon cancer HT-29 and Caco-2 cell lines (Sanaz et al., 2016). Genistein (GE), a soy-derived isoflavone, has been reported to reduce prostate (Wang et al., 2002), colon cancer (Hakkak et al., 2001; Raju et al., 2009), prostate adenocarcinoma (Joanne et al., 2009) and MDA-MB-231 breast cancer cells (Lijie et al., 2003). Previously, we reported that GE can induce apoptosis and inhibit proliferation in hepatic carcinoma PLC/PRF5 (Dastjerdi et al., 2015) and HepG 2 Cell lines (Sanaei et al., 2016). Tamoxifen (TAM) is an important drug for cancer treatment in the world. Chemoprevention trials have indicated that TAM can reduce the incidence of breast cancer (Fabian et al., 2001). Tamoxifen can induce apoptosis in human hepatocellular carcinoma (HepG2) cell line (Sebastian et al., 2004; Lee et al., 2000). Several clinical trials have reported that TAM is an active anticancer drug in ovarian cancer (Seiji et al., 2004; Hakan et al., 2007; Lee et al., 2012). Previously, we indicated that TAM can inhibit proliferation and induce apoptosis in HepG 2 cell line (Sanaei et al., 2016). It has been reported that TAM and GE combination synergistically inhibit proliferation and induce apoptosis in HepG 2 cell line breast carcinoma MDA-MB-435 cell line (Fei et al., 1999) and BT-474 human breast cancer cells (Zhiming et al., 2019).
et al., 2007). To establish that GE in combination to TAM can induce apoptosis and inhibit proliferation in human HepG 2 cell line, we investigated whether apoptosis and proliferation are altered by these compounds.

Materials and Methods

Materials

The human HCC cell line, HepG 2, was obtained from the National Cell Bank of Iran-Pasteur Institute. The cells were maintained in DMEM (Dulbecco minimal essential medium) supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/ml), and streptomycin (100 μg/ml) and cultured and incubated in 5% CO2, 95% humidified air at 37°C as reported. DMEM, GE, TAM and MTT (3-[4, 5-dimethyl-2-thiazolyl]-2, 5-diphenyl-2H-tetrazolium bromide) were purchased from Sigma (Sigma, St. Louis, MO). All other chemicals were obtained from the best available sources.

In vitro assays of HepG 2 cell growth and viability

To determine the effect of GE and TAM, the cells were seeded in triplicate in 24-well plates at the density of 1×10⁵ cells with 1 mL of medium/well and were treated with various concentrations of GE (1, 5, 10, 20 and 40 μM/lit) and TAM (1, 5, 10, 20 and 40 μM/lit), which were dissolved in dimethyl sulfoxide (DMSO); DMSO was present at 0.01–0.3%. After 24, 48 and 72 h of treatment, cell viability was assayed using methyl thiazol tetrazolium (MTT) method based on the conversion of tetrazolium dye (MTT) to a blue formazan product. The absorbance of the cell lysates in DMSO solution was read at 570 nm by a microplate reader (Bio-Rad Hercules, CA). Each assay was performed in triplicate.

Cell cycle analysis

To determine the effect of GE and TAM, the cells were seeded in triplicate in 24-well plates at the density of 5×10⁵/well. After 24 h of seeding, cells were treated with GE (20 μM) and TAM (5 μM) alone and combined except control groups received DMSO, DMSO was present at 0.01–0.3%. After 24, 48 and 72 h of drugs exposure, cells were harvested by trypsinization and then centrifuged, washed with cold phosphate-buffered saline (PBS) and resuspended in Binding buffer (1x). Finally, Annexin-V-FITC and propidium iodide (PI, Becton-Dickinson, San Diego, CA) were added and analysis was carried out according to the manufacturer’s protocol (BMS500F1/100CE Annexin-V-FITC, eBiscience, USA). All experiments were processed independently in triplicate.

Results

In vitro effects of GE and TAM on HepG 2 cell growth

Antiproliferative effects of GE and TAM (combined and alone) were evaluated by MTT assay, which indicated that these compounds can inhibit proliferation of HepG 2 cell than cells in control groups significantly. The percentage of cell proliferation of GE (1, 5, 10, 20 and 40 μM) and TAM (1, 5, 10, 20 and 40 μM) were indicated in figure 1 and 2. IC50s value for GE and TAM were observed with concentration of 20 μM after 24 h and 5 μM of TAM after 48 h respectively. The percentage of cell proliferation of GE (20μM) were 54, 40 and 36 % and of that TAM were 73, 54 and 42 % at different time periods (24, 48 and 72 h) respectively (P < 0.001). As shown in the figure 3, the combined GE (20 μM) with TAM (5 μM) decreased cell proliferation more significant than each compound alone, which decreased cell proliferation to 36, 30 and 28 after different time periods (24, 48 and 72 h) respectively (P < 0.001).

In vitro effects of GE and TAM on cell cycle progression

The results of flow cytometry assay indicated that GE with concentration of 20 μM and TAM with concentration of 5 μM (alone and combined) induced apoptosis in HepG 2 cell than cell in the control groups significantly. The percentage of apoptotic cells in GE-treated groups were 22, 34 and 48 % and of that TAM were 24, 26 and 30 % at different time periods (24, 48 and 72 h) respectively (P < 0.001). The most apoptotic cells were obtained after 72 h in GE, TAM and combined treated (GE/TAM) groups. It should be noted that GE/TAM synergistically induced
Clinical and experimental studies have reported that TAM can reduce the risk of breast cancer (Crew et al., 2017; Olver., 2016) and induce cell-cycle arrest and apoptosis of breast cancer cells (Yan et al., 2008). Recently, we reported that TAM can inhibit the growth of HepG2 cells and induced apoptosis significantly with a time- and dose-dependent manner (Sanaei et al., 2016).

In the present report, we announce that GE, TAM and combined GE/TAM inhibit proliferation and induce apoptosis in HCC HepG2 cell line. Our results are in agreement with those from other researches, showing that GE induces apoptosis and inhibits viability in Bel 7402 HCC cells (Yan et al., 2005) and also plays an important role in the prevention and inhibition of cancers such as prostate cancer, colon cancer, breast cancer, leukemia, melanoma, etc (Herman e al, 2002). Effect of GE and TAM (alone and combined) on human breast carcinoma MDA-MB-435 cells has been reported by other researchers (33) which is in line with our result. GE can induce apoptosis in HCC by different mechanism such as activation of several ER stress-relevant regulators, including caspase-12, m-calpain, GRP78 and GADD153 (Ting-Chun et al., 2007). Besides, activation of caspase-3 in GE treated HCC by which induces apoptosis and inhibits cell proliferation has been reported (Mumtaz et al., 2007). It has been reported that GE inhibits the activation of NF-κB and Akt signaling pathways in the breast and prostate cancers, both of which play a significant role to maintain a balance between cell viability and apoptosis (Sanjeev et al., 2008). Previously, we indicated that GE can act by estrogent receptor alpha in HCC PLC/PRF5 (Kavoosi et al., 2016). Primary mechanism of GE is through estrogen receptor inhibition and also non-ER-mediated pathway including modulation of signaling proteins such as calmodulin, protein kinase C (PKC), protooncogene c-myc and transforming growth factor-β (TGFβ). Furthermore, the role of c-Jun N-terminal kinase (JNK) and p38 in TAM-induced apoptotic pathway is significant (Mandlekar et al., 2001). We did not evaluate the mechanism of GE and TAM in this study. It should be evaluated the mechanism of these compounds in HepG2 cell line and other HCC cell lines.

In conclusion, our research may provide a novel approach to the treatment of HCC.
approach for the prevention and treatment of hepatocellular carcinoma and further in vivo studies to verify the efficacy of GE and TAM combination on the growth of other hepatic cell lines and also clinical evaluation of these compounds.

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