

Cytotoxic Activity of Epigallocatechin and Trans-Cinnamaldehyde in Gastric Cancer Cell Line

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Abstract

Introduction: Herbal medication is currently being utilized for treatment of numerous diseases, such as cancer which showed successful therapeutic efficacy in numerous studies. Epigallocatechin gallate (EGCG) is a compound of green tea that its role in tumor cell death has been reported. Likewise, trans-cinnamaldehyde (TC), the active ingredient in cinnamon oil showed anti-cancer properties in some previous studies. The aim of this study was to evaluate the cytotoxic effects of EGCG and TC on proliferation of gastric cancer cell line (AGS). **Methods:** AGS Cells were seeded and treated with various concentrations of EGCG and TC for 72 h and assessed for cell viability. To study the cytotoxic effect of drug in combination cases the lower doses than IC50 of EGCG and TC was utilized. The one-way analysis of variance (ANOVA) were used for statistical analysis. **Results:** various concentrations of EGCG and TC significantly inhibited the proliferation of AGS cells in dose dependent pattern. We found that in double combined cases cellular viability decreased in compared to IC50 of each single agents. Also, there were significant decrease in cellular viability in all single and double treated cases toward untreated cells ($p < 0.05$). **Conclusion:** The results of this study indicated that EGCG and TC effects on AGS cell line were significantly high and dose-dependent and might be cooperative. Double combinations of these two agents may be considered as a potential therapeutic option for gastric cancer. Further investigation should be conducted to validate these combination in gastric cancer therapy.

Keywords: Epigallocatechin- Trans-cinnamaldehyde- Gastric cancer

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Introduction

Gastric cancer is one of the main causes of cancer related death all over the worlds [1]. Treatment strategies for these patients comprisesurgery and chemotherapy. But, beneficial effects of chemotherapeutic agents are not good enough and these drugs have numerous side effects [2].

Bioactive dietary agents have displayed the capability to decrease cancer growth with noticeable immediate effects on cancer cells. Throughseparation and examination of the effective ingredients in these therapeutic agents, regarding cancer cell inhibition, a perfect mechanism of action might beassociated with animproved understanding of the profits of these natural products [3]. Recently herbal

medication utilized for treatment of numerous diseases comprising different types of cancers [4]. In this regards, trans-cinnamaldehyde (TC), the active ingredient in cinnamon oil acts as anti-inflammatory and anti-cancer agent. Likewise, TCdisplays dose-response action in inhibiting cancer cell viability that is effective strategies to counter breast cancer by reducing cancer progression [3].

Similarly, the inhibitory actions of tea catechins against cancer cell growth have been confirmed in experimental studies. Numerous mechanisms for controlling cancer signaling and metabolic pathways have been suggestedaccording to manyresearches in cell

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lines with (-)-epigallocatechin-3-gallate (EGCG), the active tea catechin. Nonetheless, the molecular basis for the suggested mechanisms and its efficacy as an anti-cancer agent *in vivo* are not evidently recognized [5]. The exposure of human stomach cancer cells to green tea catechin extract and EGCG, lead to growth inhibition and the apoptosis induction. Accordingly Morphological changes display apoptotic body in the cells exposed to EGCG and green tea catechin extract [6]. Other polyphenol of green tea, could affect development of numerous cancers [7]. So in this study the anti-cancer effects of traditional herbal medicines components; EGCG and TC in single and combined cases was examined in gastric AGS cell line.

Materials and Methods

AGS, a gastric adenocarcinoma cell line, was purchased from Pasteur Institute, Tehran, Iran. The cells were seeded in RPMI 1640 medium, with 10% (v/v) FBS and 0.1% penicillin streptomycin (at 37 °C in a humidified incubator containing 5% CO₂ atmosphere). EGCG and TC was obtained from sigma-Aldrich Company.

AGS cells (1×10^4 cells/well) were cultured on 96-well plates after 24 h, AGS cells were treated with the various concentrations of EGCG (2,5,15,25,50,60,80,90 and 100 μ M) and TC in 0.01,0.05,0.1, 0.2,0.3, 0.5, 1 and 2 mg/ml concentrations for 72 h and compared with control groups (untreated AGS cells). At the end of incubation time, cell viability was evaluated by MTT assay. MTT cell viability assay also carried out in AGS cells treated with EGCG and TC in double combinations ($0.75 \times IC_{50}$, $0.3 \times IC_{50}$, $0.2 \times IC_{50}$, and $0.1 \times IC_{50}$ doses of EGCG and TC).

Absorbance was measured by ELISA reader at the wavelength of 490 nm.

Cell Proliferation Rate = Absorbance of drug treated cells / Absorbance of untreated control $\times 100\%$ [8].

Data were presented as mean \pm standard deviation of values attained by independent tests. one-way ANOVA was utilized for multiple comparisons. p-values > 0.05 were considered as statistically significant. Statistical analysis of data was carried out with SPSS (Version 16: SPSS. Link. USA).

Results

In this study we examined cell proliferation rate of AGS gastric cancer cells against various concentrations of TC and EGCG in single and double treated cases.

As shown in Figure 1, the cytotoxic effects of EGCG and TC increased in dose dependent manner. It could be indicate that the dose response curve of both agents were partially similar.

Then, we tested the lower doses than IC₅₀ in combination of TC and EGCG on AGS cells. As shown in Figure 2, we found that in double combined cases ($0.75 \times IC_{50}$ and $0.3 \times IC_{50}$) cellular viability decreased

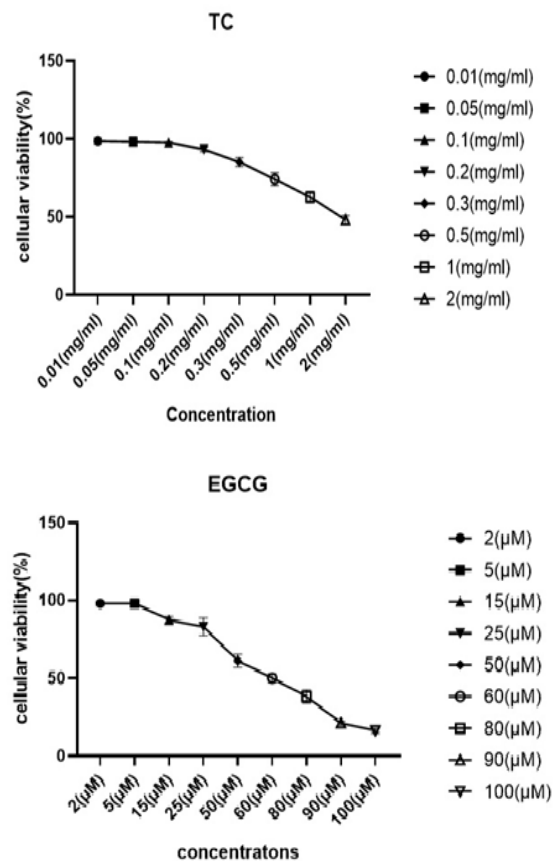


Figure 1. Cellular Viability Assay by MTT Test in AGS Cells Treated with Various Concentration of EGCG and TC. Data from independent tests presented as mean \pm standard deviation. epigallocatechin gallate; EGCG. trans-cinnamaldehyde; TC.

in compared to IC₅₀ doses of each single agents. Also, there were significant decrease in cellular viability in all single and double treated cases toward untreated cells. In double combinations there were significant higher toxicity in $0.75 \times IC_{50}$ concentrations of both EGCG and TC in compared with double combinations at $0.2 \times IC_{50}$ and $0.1 \times IC_{50}$ doses ($P < 0.05$). Also double combinations in $0.3 \times IC_{50}$ and $0.2 \times IC_{50}$ doses exerted higher growth inhibitory effects versus double combination in $0.1 \times IC_{50}$ dose ($P < 0.05$).

Discussion

There are many types of cancers such as ovarian cancer, lung cancer, and breast cancer [9-13]. Gastric cancer considered as one of the leading cause of cancer mortality world wide. indeed, numerous gastric cancer patients are identified when the tumor is at unrespectable stage. In this cases, chemotherapeutic agents in single and combined cases remains main treatment options. Previous data show that TC show inhibitory effects on cancer cell growth [3] but in many cases of cancer, favorable effects of chemotherapeutic agents are not good enough [2]. So the use of natural compound from herbal medicine could be helpful [14].

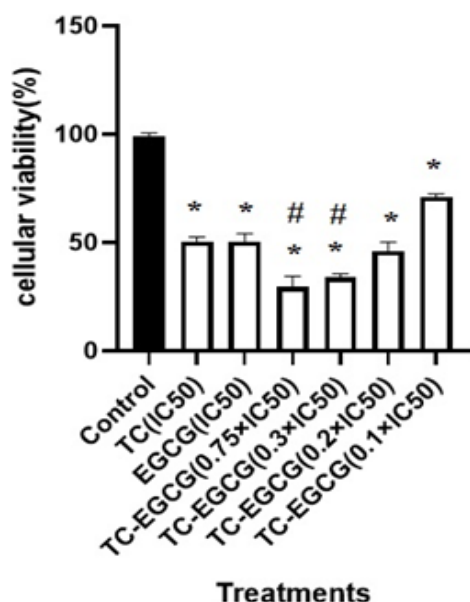


Figure 2. MTT Cell Viability Assay in AGS Cells Treated with EGCG and TC in Double Combinations ($0.75 \times IC_{50}$, $0.3 \times IC_{50}$, $0.2 \times IC_{50}$, and $0.1 \times IC_{50}$). Data are presented as mean \pm standard deviation. *Significant differences compared with untreated control cells. # Significant differences in compared to IC_{50} of each single agents. Epigallocatechin gallate; EGC. trans-cinnamaldehyde; TC.

In this regards, Cinnamaldehyde and cinnamaldehyde-derived compounds are proposed as possible potential agents as anticancer drugs [15]. Also EGCG, showed the inhibitory effects on cell growth in a various cultured cells [16].

Consequently, we examined effects of EGCG and TC in AGS cells (human gastric carcinoma). The proliferation of this cell line was inhibited with EGCG and TC in a dose-dependent manner. Also in combination of this two agents in lower concentrations ($>IC_{50}$) cell growth of AGS cells inhibited in compared with single treatments.

In similar to our results, Horie et al confirmed the synergistic impact of epicatechin with EGCG on the apoptosis induction in gastric cancer cells. In this study, caspases-3, -8 and -9 activities were assessed in EGCG-treated cells, which confirmed the caspases are engaged in the possible mechanism of EGCG action [16].

Also other study showed the potential efficacy of Cinnamaldehyde as a new antitumor agent. The mechanisms of action include the regulation of apoptosis adhesion and invasion related genes [17].

Chiang et al in a recent study on breast cancer cell line and xenograft animal models that treated with various concentrations of visfatin combined with CA and FK866 (a visfatin inhibitor) tested the cell toxicity. In the breast cancer cell and the xenograft animal model, visfatin enhanced proliferation-related protein, nevertheless combination with CA or FK866 significantly decreased visfatin-induced carcinogenic impacts. They indicate that TC decreased visfatin-related breast cancer progression.

In describing the possible mechanism of increased

cytotoxicity of TC and EGCG could be indicated that these two agents might be acts in cooperative manner in induction of cell death.

In this regards it has been reported that EGCG repressed gastric tumour growth through inhibiting Wnt/ β -catenin signalling. Indeed, EGCG decreased gastric cancer cell proliferation [7]. Also TC promote cancer cell death and apoptosis induction [17]. It seem that these effects was increased in our studied combinatorial cases.

To the best of our knowledge we didn't find any more related studies and these combination were evaluated in this study for the first time. Further studies should be conducted to verify the possible mechanisms which engaged in cytotoxic effects of EGCG and TC in combined cases.

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