Epicatechin Enhances Anti-Proliferative Effect of Bleomycin in Ovarian Cancer Cell

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Abstract

Background: Bleomycin (BLM) is an anti-cancer drug widely used in the treatment of cancer. BLM causes several side effects related to DNA and cellular damage. The aim of this study was investigated the effects of tea polyphenol epicatechin on anti-proliferative effects induced by bleomycin in human normal skin and human ovarian cancer cells.

Materials and Methods: Human ovarian cancer cell (SKOV-3) and human non-malignant fibroblast cell (HFFF2) were treated with epicatechin at various concentrations (10, 25 and 50 µM) and BLM alone and with their combinations, further their effects on cell viability were evaluated.

Results: The combined treatment of epicatechin with BLM enhanced significantly inhibition of cell growth in comparison to BLM alone in cancer cell. Epicatechin enhanced significantly cytotoxicity induced by BLM with 83% at dose 50 µM, while it was 92% in BLM-treated cells. Epicatechin was not showed any cytotoxicity on HFFF2 cells.

Conclusion: Study suggests that epicatechin chemosensitize the ovarian cancer cell to BLM-induced growth inhibition without any toxicity on normal cell.

Keywords: Bleomycin; Epicatechin; Tea; Anti-proliferation; MTT; Ovarian cancer

Introduction

Bleomycin is a family of glycopeptide antibiotics which uses widely for treatment of different cancers. BLM binds to DNA (not RNA) through its aminoterminal peptide, and generates free radicals that attack to DNA and producing single and double strand breaks (1). DNA damage by BLM depends on metal ions and oxygen. BLM is forming a complex with metal mainly iron (Fe (II)), this complex with a one-electron reductant activates BLM. Activated BLM produces superoxide and hydroxide free radicals that cleave DNA (2, 3). BLM acts as cell arrest in the G2-M phase of the cell cycle (4). BLM causes extensive damage to DNA similar to that generated by ionizing radiation; BLM is known as a radiomimetic drug (5). Therefore BLM causes DNA cleavage and fragmentation leading finally to cellular apoptosis, necrosis and deaths. However, the antineoplastic drug bleomycin is now a second-line therapy for certain solid tumors (6), it causes several side effects such as nausea, vomiting, fever (often with rigors) and occasional allergic type reactions, but pulmonary toxicity are is a major adverse effect (7). This side effect was widely studied in animal models and it is related to oxidant-induced inflammatory and fibrotic lesions in the lung through oxidative stress (5, 8).

Green tea polyphenols are potent antioxidants (9). Green tea is containing flavan-3-ols, commonly known as catechins, which include (−)-epigallocatechin-3-gallat (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG) and (−)-epicatechin(EC) (10).

However, several experiments demonstrated the inhibitory action of tea components against carcinogenesis in animal models such as lung, skin, breast and stomach cancers (11-14).
The antitumor effects of these green tea catechins have been studied at cellular level, and the catechins were found to be induced apoptosis and cell cycle arrest. Potential mechanisms have also been suggested to include anti-oxidative activity, inhibition of enzymes related to tumor promotion such as cyclooxygenase and lipoxygenase, inhibition of activator protein-1, inhibition of angiogenesis, activation of p53 tumor suppressor protein and inhibition of telomerase and metalloproteinases activity (11-17).

The present study was investigated the effects of tea polyphenol epicatechin on anti-proliferative effects induced by bleomycin in human non-malignant fibroblast cell and human ovarian cancer cells.

Materials and Methods

Chemicals
Epicatechin (Fluka, USA) and Bleomycin (Kwality, India) were dissolved in sterile water at stock solution and diluted with RPMI 1640 medium. 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma (USA).

Cell culture
Human ovarian cancer (SKOV-3) and human skin fibroblast (HFFF2) cells were got from the Pasture Institute of Iran and cultured at 37 °C and 5% CO2 in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco, Paisley, UK) supplemented with 10% fetal bovine serum (FBS) and 100 µg/ml penicillin-streptomycin (Gibco). Experiments on cells were performed in the exponential growth phase.

Cell anti-proliferation assay
Untreated and treated SKOV-3 and HFFF2 cells were subjected to cell proliferation assay using MTT to quantify the metabolic activity to cleave tetrazolium salts (18, 19). Cells (20,000) were seeded in 96-well plates. After 24 h incubation, cells were treated with various concentrations of epicatechin (10, 25 and 50 µM) and incubated for 2 h at 37 °C and 5% CO2. Also, RPMI 1640 culture medium was set as negative control, and only culture medium without any cells was set as blank control. After incubation, BLM was added at 10 µg/mL to each well. All testing and control groups were repeated in triplicate. At 48 hours of culture, 20 µL MTT (5 mg/mL in phosphate buffer saline) was added to every well, and culturing was continued for 4 hours. Then, culture supernatant was discarded and replaced by 150 µL isopropanol (0.1%HCl), and the cell plates were shaken for 30 minutes. Finally, the absorbance of every culture well was read on an ELISA Reader (Bioteck, USA) at 490/630 nm.

Statistical analysis
Data were presented as mean ± standard deviation (SD) of three independent experiments. Data were compared and the differences were considered significant if the p value <0.05.

Results
Cell viability of SKOV-3 and HFFF2 cells treated with the doses of epicatechin in combination with BLM or alone was determined by MTT colorimetric assay. To examine the effect of epicatechin on ovarian cancer, SKOV-3 cells were treated with various concentrations (10, 25 and 50 µM) of epicatechin for 48h. Epicatechin alone was not showed any inhibitory effects on SKOV-3 cells. BLM alone was not showed any significantly inhibition effect on growth of SKOV-3 cells, however, epicatechin in combination with BLM was exhibited a dose-dependent manner in growth inhibitory on ovarian cancer cells (Figure 2).

Epicatechin enhanced significantly cytotoxicity with 83% at dose 50 µM (p<0.01). Other doses of epicatechin (10 and 25µM) were not significantly exhibited any enhancement cytotoxicity in combination with BLM. HFFF2 cell was selected as a normal human skin fibroblast for assessment of any cytotoxicity induced by BLM in combination with epicatechin. Anti-proliferation on HFFF2 cells was not observed at various concentrations of epicatechin.
alone. Epicatechin was not showed any significantly enhancement cytotoxicity in combination with BLM on HFFFE2 (Figure 3).

**Discussion**

The recent preclinical success of epicatechin tea polyphenol in growth suppression of cancer cells suggested the potential uses of dietary substances in the treatment of cancers in combination of bleomycin as a well known anticancer agent. It is may interested to research by combining phytochemicals with chemotherapy, it is possible to improve the effectiveness of the cancer treatment and minimizing toxicity (20).

![Figure 3. Anti-proliferative effects of epicatechin (EP) (10, 25 and 50 µM) with bleomycine (BLM) (10 µg/mL) on human normal skin cell (HFFFE2). Non significant was observed between groups](image-url)

We compared these effects with the cytotoxic chemotherapeutic agent BLM, alone or in combination with epicatechin for development of less toxic treatment strategies for ovarian cancer cell. Epicatechin acted as anti-carcinogenic effects against oxidative stress and protected promotion phase of cancer in liver epithelial cell (21). Several studies showed that epicatechin exhibited anti-tumor affects (22-24). It is clear that anti-cancer agent such as BLM is producing oxidative stress leading to cellular toxicity, and then epicatechin may have a contraindication with BLM on cancer cells. Our finding showed that epicatechin exhibited enhancement toxicity in combination with BLM on ovarian cancer cell, while it was not showed any cytotoxicity on normal human skin cell. These results are interesting for usage of this tea component in cancer chemo-therapy. There are proposed two mode actions for epicatechin, first it acts as anti-cancer on tumor cells, and secondly epicatechin enhances the efficacy of BLM on inductions of growth inhibition in human ovarian cancer cell. Epicatechin acted inhibiting the binding of NF-κB to DNA and inhibited cellular growth on Hodgkin's lymphoma, this inhibition was not associated with epicatechin antioxidant activity (25). Epicatechin was found to have cytotoxic activity, and cells treated with this compound had fragmented nuclei, fragmented DNA, and underwent apoptosis on SW480 human colon cancer (26). Saha et al. reported that the combination of curcumin with epicatechin significantly increased the inhibition of cell growth compared with curcumin or epicatechin alone. Epicatechin may enhance the incorporation of curcumin into human lung cancer cell lines PC-9 and A549 (27). It is hypothesized that two above mentioned actions participated a chemo-sensitive effect of epicatechin on cytotoxicity induced by BLM.

**Conclusion**

The present study shows that epicatechin, a naturally occurring tea polyphenolic agent, could enhance the anti-proliferation of bleomycin on human ovarian cancer cells SKOV-3. It was also shown that epicatechin did not caused any inhibition on normal human skin cells HFFFE2. As evidenced from the results, epicatechin may be a powerful candidate in the development of therapeutic agents for ovarian cancer therapy with bleomycin. On the basis of these results, further investigations and in vivo must be performed to determine the possible clinical applications of these combinations.

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