

Effects of Curcumin in Combination with Doxorubicin in Human Colorectal Cancer Cell Line

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Abstract

Background: Colorectal cancer (CRC) is one of the main cause of cancer related death worldwide. New therapeutic strategies are required for CRC. Anthracycline drugs such as doxorubicin (DOX) remain one of the most active wide-spectrum and cost-effective drugs in cancer therapy. However, colorectal cancer (CRC) cells are inherently resistant to anthracyclines. Curcumin, the active component and yellow pigment, has been considered as anti-cancer agents with anti-proliferation, anti-invasion, and anti-angiogenesis properties. Previous data show that curcumin may played main role as therapeutic agent for CRC. We aimed to assess the possible sensitizing effects of curcumin in HCT-116 CRC cells treated with DOX. **Methods:** HCT-116 cells were treated with different doses of curcumin and DOX in increasing concentrations and cytotoxicity were evaluated after 48 h by Water Soluble Tetrazolium Salts(WST-1) method. In double combination treatments (48 h), the mentioned concentration were utilized; D (Curcumin; 20 μ M; DOX; 5 μ M) C (Curcumin; 10 μ M; DOX ; 2.5 μ M), B (Curcumin; 5 μ M; DOX; 1 μ M) and A (Curcumin; 2.5 μ M; DOX; 0.5 μ M). **Results:** HCT-116 cells treatments with various concentrations of single agents (DOX and curcumin) decreased the cellular viability in a dose-dependent pattern. Here, we show that treatment of HCT-116 CRC cells with curcumin increases the efficacy of DOX-induced death in HCT-116 cells. Curcumin treatments also results in higher cytotoxicity of DOX and the cell death percent in compared to higher doses in single treatments. **Conclusion:** It could be concluded that curcumin could acts as chemosensitiser towards the DOX therapy. So it might be used as adjuvant therapy to enhance DOX sensitivity in CRC cells.

Keywords: Colorectal cancer- curcumin- doxorubicin

Asian Pac J Cancer Biol, 3 (4), 89-92

Submission Date: 10/02/2018 Acceptance Date: 12/03/2018

Introduction

Colorectal cancer (CRC) is one of the main leading causes of cancer related mortality worldwide [1]. Although the patients survival has recently increased due to improved surgery, local radiotherapy and chemotherapy, but the high rate of metastatic patients prognosis are not good [1-2]. Indeed, one of the main goals in cancer therapy is to predict response to chemotherapeutic drugs by drug response assays [3]. Anthracycline drugs including doxorubicin

(DOX) considered as furthestmost active wide-spectrum and Inexpensive drugs in cancer treatment. However, CRC cells are characteristically resistant to anthracyclines [4]. Indeed cancer cells shows resistance toward anti-cancer agents in major cases [1-2]. On the other hands, numerous routine chemotherapeutics have adverse side effects; dietary manipulations might have an important role in the prevention of numerous human cancers. Curcumin the yellow pigment in turmeric has been extensively utilized in the Asian countries [5]. Previous data has been revealed that curcumin prevent the growing of transformed cells

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and colon carcinogenesis at the beginning and progression phases in carcinogen-induced models [6-7]. We aimed to evaluate the possible sensitizing effects of curcumin in HCT-116 CRC cells treated with DOX.

Materials and Methods

Cell lines and Cell Cultures

Human HCT-116 cells were purchased from the Pasteur Institute (Tehran, Iran). Cells were maintained in growth medium consisting of Dulbecco's modified Eagle's medium [DMEM (BioWest, France)] supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic (BioWest, France) at 37°C and 5% CO₂. Cells were passaged utilizing trypsin/EDTA. Curcumin was purchased from Sigma Chemical Co. (USA), dissolved in 100% ethanol, and then diluted with the DMEM. Doxorubicin hydrochloride purchased from sigma-Aldrich (USA) and solubilized in pure water.

Treatments and Cellular Viability Assay

Cells were treated with curcumin and DOX (viability in response to curcumin and DOX was assessed by WST-1 after 48 h. Briefly, cells were dispersed by trypsin-EDTA treatment and 1×10^4 cells/ml, re-suspended in DMEM containing 10% FBS and seeded into 96-wells culture plates with 3 replicates. After 24 h of plating, treatments was performed for 48 h in the absence (control) or presence of DOX (1,2.5, 5,10,20 and 40 μ M) and curcumin (10, 20, 50, 75 and 100 μ M) in single treatments. The concentration of double combination were as below in A to E groups.

D (Curcumin; 20 μ M;DOX;5 μ M) C (Curcumin; 10 μ M; DOX ; 2.5 μ M), B (Curcumin; 5 μ M;DOX; 1 μ M) and A (Curcumin; 2.5 μ M; DOX; 0.5 μ M).

The E group (control) considered as control which include the untreated cells had any exposure to DOX and curcumin.

At the end of the 48 h incubation period, the reaction was terminated by adding 10 μ l of WST-1 stock to each well. The reaction was allowed to proceed for 3–4 hours at 37°C. Cell viability was assayed according to the cleavage of the tetrazolium salt, water-soluble tetrazolium-1 (WST-1), to dark red formazan. Absorbance was read at 420 nm with a reference wavelength > 650 using the enzyme-linked immunosorbent assay microplate reader. All values were compared to the related controls. All assays were performed with triple replicates.

Statistical analysis

Data shown as the mean \pm SD. The differences among more than two means were evaluated by one-way analysis of variance (ANOVA) with SPSS software, V. 10.0 (SPSS Inc, Chicago, Illinois). $P < 0.05$ considered as significance level.

Results

Effects of DOX on cell viability

The cytotoxic effects of the single treatments of DOX and curcumin after 48 h of exposure in CRC cell line plotted as the percentage of viable cells versus control cells (Figure 1). As presented in Figure 1, the exposure of HCT-116 cells to various concentrations of single agents (DOX and curcumin) decreased the cellular viability in a dose-dependent pattern.

Cytotoxic effects of DOX in combination with curcumin on HCT-116 cells

Different concentrations of DOX in combination with curcumin were selected according to the initial tests mentioned in the methods to evaluate the cytotoxic effects of drug combinations on the HCT-116 cell line.

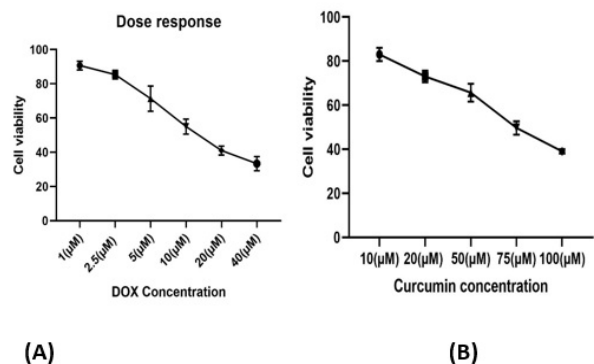


Figure 1. Dose Response Curve of DOX (A) and Curcumin (B) Treatments in HCT-116 Cells in the Concentrations of 1,2.5, 5, 10, 20 and 40 μ M of DOX and 10, 20, 50,75and100 μ M of Curcumin; DOX, doxorubicin (μ M).

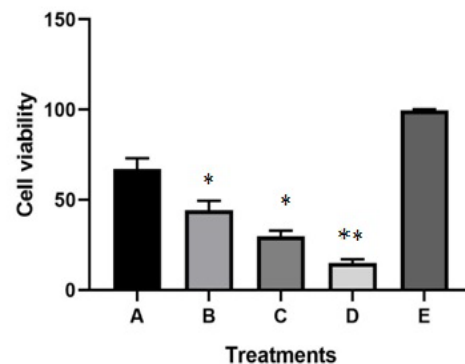


Figure 2. Results of WST-1 (cellular viability assay) in Combination Cases of Curcumin and DOX in Different Concentrations that Presented as Mean \pm standard deviation; ** show the significant difference in compared to A, B, C and E group ($p < 0.05$). * show the significant differences in compared to A and E group ($p < 0.05$); D, Curcumin; 20 μ M;DOX;5 μ M (concentrations); C, Curcumin; 10 μ M;DOX ;2.5 μ M (concentrations); B, Curcumin; 5 μ M;DOX;1 μ M (concentrations); A, Curcumin; 2.5 μ M;DOX;0.5 μ M (concentrations); E, Untrated HCT-116 cells (Control).

WST-1 results from double combination cases are presented in Figure 2.

In the case of double combination treatments, drug concentrations were; D (Curcumin; 20 μ M; DOX; 5 μ M) C (Curcumin; 10 μ M; DOX ; 2.5 μ M), B (Curcumin; 5 μ M; DOX; 1 μ M) and A (Curcumin; 2.5 μ M; DOX; 0.5 μ M). The E group (control) was the untreated cells which had any exposure to DOX and curcumin.

The DOX in combination with curcumin showed increased cytotoxicity compared to higher concentrations (the doses, which led to 50% of cell killed in single treatments) of each single agents. The D group had higher cytotoxic effects in compared to A, B, C and E groups ($p < 0.05$). In addition, the C and B group showed increased toxicity in compared to A and E groups ($p < 0.05$). Indeed, in combination cases in the low concentrations of DOX and curcumin, the cellular viability were decreased in compared to each single drug treatments in the concentration which led to 50% of cell toxicity [C and D treatments had significant elevated toxicity versus to each single treatments ($p < 0.05$)].

Discussion

Amongst diverse ranges of disorders, cancer has always considered as the furthestmost important condition, which affected many individuals word width. In this regards, different cancer therapeutics applied to overcome cancer cell growth [3-8,16]. CRC considered as one of the commonest cancers all over the worlds. In the face of innovative treatment strategies, CRC is infrequently cured because of recurrence. Numerous studies have utilized curcumin, as anti-cancer agents in numerous types of cancer, such as CRC [17]. Since, Anthracycline drugs such as DOX remain some of the most active wide-spectrum and cost-effective drugs in cancer therapy and CRC cells are resistant to anthracyclines, we evaluated the effects of curcumin in combination with DOX in CRC cells.

Based on our data DOX and curcumin in the dose dependent manner reduced the cellular viability. In combination cases in the lower concentrations of DOX and curcumin, the cellular viability were decreased in compared to each single drug treatments in the concentration which led to 50% of cell killed.

Our study assess the curcumin effects in combination with DOX, to evaluate how curcumin increase the cytotoxic effects of DOX in CRC cells. For this elevated cytotoxic effects in combination cases, it could be proposed with that fact which described by Chauhan et al. in this study , they indicated that curcumin should be considered as a harmless, non-toxic chemotherapeutic drug [7] which impedes lipooxygenase activity , impeding the cytochrome P-450 enzyme activity and promotion/progression stages of carcinogenesis. The anti-cancer properties of curcumin has been associated with arrest of cancer cells in S, G2/M cell cycle phase and apoptosis activation [7].

Recently researchers indicated that curcumin in combination with DOX was effective in sensitizing cancer cells that are resistant to DOX and in decreasing cancer cells viability. Curcumin has also been shown

to induce the immuno-, hepato-, and cardioprotective characteristics of DOX [18] which is similar to our data.

Also, Meiyanto et al utilized curcumin in combination with DOX that increased the sensitivity of resistant MCF-7 cells to DOX, which was comparable to our results [19].

Other study showed that two drug (curcumin+DOX) loaded in a single nanoparticle formulation decrease the drug resistance by effects on gradual mRNA expression of MDR1 and Bcl-2 in CML blast-like K562 cells [20].

In addition, Huang et al reported that curcumin could increase the impacts of irinotecan on CRC cells by effect on cell viability and apoptosis, which is similar to our results [21].

In conclusion , it could be deduced that curcumin as natural agents can affect main carcinogenic pathways without demonstrating noticeable adverse impacts, which might be considered as perfect chemosensitizer agents in combination with DOX. In this study, we confirmed the cytotoxic effects of dietary agent's curcumin for chemosensitizing of DOX.

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