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Original article

Anti-cancer and cardioprotective effects of indol-3-carbinol in doxorubicin-treated mice

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ABSTRACT

Doxorubicin (DOX) is a broad-spectrum antitumor antibiotic used in treatment of cancer. Its effect may be complicated by increased risk of cardiotoxicity. It was suggested that natural compounds with anti-cancer properties can be used in combination with DOX to decrease its dose and side effects. Indole-3-carbinol (I3C) is one of the phytochemicals that was shown to have anti-cancer effect. Our aim was to detect the possible chemosensitizing effects of I3C in DOX-induced cytotoxicity and the possible cardioprotective effects of I3C in DOX-induced cardiotoxicity. One hundred mice were divided into five equal groups: Control untreated group, solid Ehrlich carcinoma (SEC), SEC + DOX, SEC + I3C, SEC + DOX + I3C. Tumor volume, serum creatinine kinase and lactate dehydrogenase were measured. Also, tissue malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), sphingosine kinase-1 (SphK1) activity and interleukin-6 (IL-6) were determined. Parts of the tumor and cardiac tissues were subjected to histopathological examination. DOX or I3C alone or in combination induced significant increase in tumor CAT and SOD with significant decrease in tumor volume, tumor MDA, SphK1 activity and IL-6 and alleviated the histopathological changes with significant increase in the apoptotic index and significant decrease in tissue bcl2 compared to SEC group. Also, DOX induced cardiotoxicity which was ameliorated by I3C. In conclusion, DOX/I3C combination had a better effect than each of DOX or I3C alone against SEC in mice with marked improvement of the cardiotoxicity induced by DOX.

1. Introduction

Doxorubicin (DOX) is one of the most potent broad-spectrum antitumor antibiotics. It can be administered as a single agent or in combination with other chemotherapeutic agents for treatment of variety of cancers, including leukemia, lymphoma and solid tumors. Its cytotoxic effects on malignant cells, however, are complicated by an increased risk of cardiotoxicity [1,2]. So, it has become increasingly important to find pharmacological remedies to protect against this serious side effect. In an attempt to minimize DOX effective chemotherapeutic dose and thereby its side effects, a variety of approaches were investigated including the search for natural compounds with anticancer properties that can be used in combination with DOX [3,4].

Epidemiological and dietary studies have provided a link between dietary intake of cruciferous vegetables and lowered cancer risks. Considerable evidence attributes this chemopreventive effect to the antitumor activity of a common phytochemical, indole-3-carbinol (I3C), and its metabolites. They have been shown to suppress the proliferation of various cancer cell lines by targeting a wide spectrum of signaling pathways that regulate hormonal homeostasis, cell cycle progression, and cell proliferation [5,6]. Moreover, I3C inhibited tumorigenesis in mammary glands, liver, lung, and gastrointestinal tract in different animal models. These preclinical findings demonstrate the value of I3C in cancer prevention and therapy, which has led to its human trials in cervical dysplasia, breast cancer and recurrent respiratory papillomatosis [7]. The aim of this study was to detect the possible chemosensitizing effects of I3C in DOX-induced cytotoxicity and its possible cardioprotective effects on DOX-induced cardiotoxicity.

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2. Materials and methods

2.1. Drugs used

Doxorubicin was commercially available in powder form for injection purchased from Carlo Erba, Turkey. It was dissolved in normal saline and administered by intraperitoneal injection in a dose of 4 mg/kg body weight once weekly for 4 weeks [8]. I3C was purchased from Sigma Aldrich Co. and administered daily orally in diet.

2.2. Solid Ehrlich carcinoma (SEC) tumor model

1×10^6 of Ehrlich carcinoma cells (ECC) obtained from the pharmacology and experimental oncology unit of the national cancer institute, Cairo University, Egypt were implanted subcutaneously into the right thigh of the hind limb of mice. A palpable solid tumor mass was developed within 12 days [9].

2.3. Classification of animals

In this study, we used one hundred BALB/c mice weighing about 20–25 g. All the experiments were conducted according to the National Research Council's guidelines. Animal handling was followed according to Helsinki declaration of animal ethics. The animals were divided into 5 equal groups of twenty mice each as follows:

Group (1): is the normal control group.

Group (2): Ehrlich tumor cells were implanted subcutaneously into the right thigh of the hind limb of mice [9].

Group (3): Doxorubicin was given by intraperitoneal injection on days 0, 7, 14, 21 after implantation of ECC [8].

Group (4): Mice were put on diet containing 2000 ppm I3C one week before and continued for 6 weeks after implantation of ECC [10].

Group (5): Mice were put on diet containing 2000 ppm I3C one week before and continued for 6 weeks after subcutaneous implantation of Ehrlich tumor cells concomitantly with intraperitoneal injection of doxorubicin on days 0, 7, 14, 21 after implantation of ECC.

2.4. Assessment of the time-course effects of different treatments on tumor volume (TV) of SEC

Tumor volumes were recorded from the start point at 15th day post-implantation and thereafter every 5 days till the 40th day post-implantation just prior to scarification of mice using a Vernier caliper. Tumor volume (V) was calculated as $V\,(\mathrm{mm}^3) = (a^2 \times b)/2$, where a (small diameter), and b (large diameter) are perpendicular, expressed in millimeters (mm).

At the end of the study, all mice were sacrificed. Blood samples were collected and centrifuged to measure serum creatinine kinase (CK-MB) according to Hess et al. [11] and serum lactate dehydrogenase (LDH) according to Buhl and Jackson [12]. Parts of the heart and tumor tissues were homogenized for determination of tissue catalase (CAT) according to Higgins et al. [13], tissue malondialdehyde (MDA) according to Uchiyama and Mihara [14], tissue superoxide dismutase (SOD) according to Marklund and Marklund [15], tissue interleukin-6 (IL-6) using ELISA kits purchased from Sigma chemical Co. according to the instructions of the manufacturer and tumor tissue sphingosine kinase 1 (SphK1) activity using ELISA kits (Echelon Biosciences Inc., K-3500) according to the manufacture's protocol.

2.5. Assay of tissue caspase-3 activity

A piece of the tumor tissue was homogenised and proteins were extracted and stored at −80 °C. 100 μg of tumor tissue extracts in the assay buffer (50 mM HEPES, pH 7.4, 100 mM NaCl, 0.1% CHAPS, 10 mM dithiothreitol, 1 mM EDTA,10% glycerol) was added to 100 μM of the peptide substrate N-acetyl–Asp–Glu–Val–Asp–ρ-nitroanilide (Ac–DEVD–ρNA) and incubated at 37 °C for 1 h. Cleavage of the substrate was monitored every 30 min up to 2 h at 405 nm and the enzyme activity was expressed as nmol/min/mg protein.

2.6. Histopathological and immunohistochemical examination

Parts of the tumor and cardiac tissues were prepared and stained with hematoxylin and eosin (H&E) and Mallory's trichrome stain for histopathological study. Apoptotic index was measured as aggregate percentage of apoptotic cells and/or apoptotic bodies per total number of cells (1000 cells counted) in 10 randomly selected high power fields. The morphological criteria for apoptotic bodies were applied according to Staunton and Gaffney [16].

Assessment of tumor tissue bcl2 was carried out in formaline-fixed, paraffin embedded SEC sections using monoclonal antibodies against bcl-2 that were purchased from Zymed Laboratories Inc., USA. Bcl-2 was considered positive if the tumor cells showed cytoplasmic or perinuclear localization of immunoreactivity. This was expressed as follows: (++++): the largest number of cells showing positive staining for bcl2; (+++): intermediate number of bcl2-positive cells; (++): indicates lower number of cells showing positive staining for bcl2 [17].

2.7. Statistical analysis

The data obtained were subjected to one way ANOVA and Tukey's multiple comparison test. Data were presented as mean ± S.E.M. Differences between the means of different groups were considered significant at a level of p-value less than 0.05.

3. Results

3.1. Effect of different treatments on tumor volume

DOX and/or I3C resulted in significant decrease in tumor volume compared to SEC group. This decrease was significant in DOX/I3C combination group compared to the groups that received either DOX or I3C alone (Fig. 1).

3.2. Effect of different treatments on tumor tissue antioxidant status

Subcutaneous implantation of ECC resulted in significant decrease in tissue CAT and SOD with significant increase in tissue MDA compared to the control untreated group. DOX and/or I3C resulted in significant increase in tissue CAT and SOD with significant decrease in tissue MDA compared to SEC group. The increase in tissue CAT and SOD and the decrease in tissue MDA were significant in DOX/I3C combination group compared to the groups that received either DOX or I3C alone (Table 1).

3.3. Effect of different treatments on tumor tissue IL-6

Subcutaneous implantation of ECC resulted in significant increase in tissue IL-6 compared to the control untreated group. DOX and/or I3C resulted in significant decrease in tissue IL-6 compared to SEC group. The decrease in tissue IL-6 was significant in DOX/I3C