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Prevention of doxorubicin (DOX)-induced genotoxicity and cardiotoxicity: Effect of plant derived small molecule indole-3-carbinol (I3C) on oxidative stress and inflammation

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ABSTRACT

Doxorubicin (DOX) is an anthracycline group of antibiotic available for the treatment of broad spectrum of human cancers. However, patient receiving DOX-therapy, myelosuppression and genotoxicity which may lead to secondary malignancy and dose dependent cardiotoxicity is an imperative adverse effect. Mechanisms behind the DOX-induced toxicities are increased level of oxidative damage, inflammation and apoptosis. Therefore, in search of a potential chemoprotectant, naturally occurring glucosinolate breakdown product Indole-3-Carbinol (I3C) was evaluated against DOX-induced toxicities in Swiss albino mice. DOX was administered (5 mg/kg b.w., i.p.) and I3C was administered (20 mg/kg b.w., p.o.) in concomitant and 15 days pretreatment schedule. Results of the present study showed that I3C appreciably mitigated DOX-induced chromosomal aberrations, micronuclei formation, DNA damage and apoptosis in bone marrow niche. Histopathological observations revealed that DOX-intoxication resulted in massive structural and functional impairment of heart and bone marrow niche. However, oral administration of I3C significantly attenuated DOX-induced oxidative stress in the cardiac tissues as evident from decreased levels of ROS/RNS and lipid peroxidation, and by increased level of glutathione (reduced) and the activity of phase-II antioxidant enzymes. Additionally, administration of I3C significantly ($P < 0.05$) stimulated Nrf2-mediated activation of antioxidant response element (ARE) pathway and promoted expression of cytoprotective proteins heme oxygenase 1 (HO-1), NAD(P)H:quinine oxidoreductase 1 (NQO1) and GSTπ in bone marrow and cardiac tissues. In connection with that, I3C significantly attenuated DOX-induced inflammation by downregulation of pro-inflammatory mediators, viz., NF-kβ(p50), iNOS, COX-2 and IL-6 expression. Moreover, I3C attenuate DOX-induced apoptosis by up-regulation of Bcl2 and down-regulation of Bax and caspase-3 expression in bone marrow cells. Thus, this study suggests that I3C has promising chemoprotective efficacy against DOX-induced toxicities and indicates its future use as an adjuvant in chemotherapy.

1. Introduction

Doxorubicin (DOX) also known as adriamycin is an anthracycline antibiotic widely used in the treatment of several types of human malignancies including haematological malignancies, solid tumors, soft-tissue sarcomas and breast carcinoma [1,2]. The therapeutic potential of DOX is achieved through the processes of intercalating into DNA, inhibiting topoisomerase II, preventing DNA and RNA synthesis [3]. However, the clinical use of DOX is limited due to the development of myocardial toxicity in patients as well as cytotoxic effects to normal cells, leading to unwanted side effects which may substantially impact patient health and quality of life [4,5]. In addition, nausea, vomiting, neutropenia, alopecia and arrhythmias are the acute adverse effects of DOX-therapy [6]. In connection with that, DOX-induced acute cardiotoxicity is approximately 11% during and within 2–3 days of its administration [7,8]. Metabolism of DOX in our body significantly increased the production of free radicals and the occurrence of lipid peroxidation [9,10] as well as depletion of antioxidants and sulfhydryl groups [11,12]. According to the present knowledge, the mechanisms responsible for DOX-induced cardiotoxicity appears to be multifactorial, involving increased lipid peroxidation, oxidative stress, DNA/RNA damage, endoplasmic reticulum-mediated apoptosis and disturbance of calcium homeostasis [13]. Additionally, the metabolism of DOX through NADPH-cytochrome P-450 enzyme leads to the formation of superoxide anions and hydroxyl radicals, which in turn cause injury to cellular membranes. Moreover, DOX can stimulate inducible nitric oxide synthase (iNOS) enzyme, increasing the production of nitric oxide (NO), which is associated with dilated cardiomyopathy and congestive

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heart failure [14]. Due to great importance of DOX in cancer chemotherapy, it is essential to reduce its toxicity to normal cells, a goal that can be achieved by concurrent administration of free radical scavenging agents such as antioxidants [15]. Thus, attenuating oxidative stress, inflammation and apoptosis is a potential therapeutic strategy against DOX-induced toxicity [16]. We therefore postulated that activation of endogenous antioxidant pathways might be a potential complement for DOX-induced toxicity. Thus, inhibition of apoptosis, attenuating oxidative stress as well as pro-inflammatory mediators, viz., nuclear factor-kβ (NFkβ), iNOS, cyclooxygenase-2 (COX-2) and interleukin-6 (IL-6) expression is an impending therapeutic approach against DOX-induced toxicity.

Indole-3-carbinol (I3C), a naturally occurring glucosinolate breakdown product found in cruciferous vegetables such as cabbage, broccoli and Brussels sprouts [17,18]. It is one of the phytochemicals that was shown to poses potent anti-estrogenic [19] or apoptosis-inducing property in cancer cells [20]. Earlier studies reported that I3C has the ability to amplify some antioxidant enzymes activity such as hemoxygenase-1 and glutathione transferase [21]. Moreover, I3C was shown to possess chemopreventive activity against benzo[a]pyrene-induced mouse forestomach carcinogenicity [22] as well as anti-tumor activities via interference with a variety of signal transduction pathways involved in cell survival [23]. Additionally, I3C attenuated lipid peroxidation by normalizing the activities of antioxidant enzymes in host organs. It was also reported that I3C has the ability to stabilized cell membrane and reduced leakage of myocyte injury marker enzymes [24]. Moreover, I3C modulated cell death mediators through down-regulating sphingosine kinase 1 (SphK1) activity and inflammatory mediators along with mitigated histological perturbations [14]. These facts motivated us to evaluate the protective role of I3C against DOX induced toxicities through inhibition of apoptosis, modulation of Nrf2/ARE and inflammatory response pathway in Swiss albino mice.

2. Materials and methods

2.1. Experimental animals

In this study, Adult (5–6 weeks old) Swiss albino female mice (25 ± 2 g b.w.), bred in the animal colony of Chittaranjan National Cancer Institute (Kolkata, India) were used. They were maintained at control temperature (23 ± 2 °C) and humidity (55 ± 10%) under alternating light and dark conditions (12 h/12 h). Animals were fed with standard food pellet diet (EPIC rat and mice pellet from Kalyani Feed Milling Plant, Kalyani, West Bengal, India) and drinking water was provided *ad libitum*. All procedures for animal experimentation used were approved by the Institutional Animal Ethics Committee (CPCSEA Reg. No.-1774/GO/RBi/S/14/CPCSEA, India)].

2.2. Chemicals

Indole-3-Carbinol was purchased from Sigma-Aldrich Chemicals Private Limited, Bangalore, India (Purity $\geq$ 96%). Doxorubicin was obtained from Cipla LTD, Verna, Goa, India. in situ cell death detection kit, AP was purchased from Roche Diagnostics India Private Limited, Bangalore, India. Nrf2, Keap1, HO1, NQO1, NFkβ(p50), iNOS, COX-2, IL-6, anti-mouse IgG-HRP, anti-goat IgG-HRP, anti-rabbit IgG-HRP and Luminol were bought from Santa Cruz Biotechnology (Texas, USA). GST-π was purchased from Cell Signaling Technology (Danvers, USA). GAPDH and Histone H3 were purchased from Novus Biologicals (Colorado, USA). All other chemicals not specified were obtained from Sigma-Aldrich Chemicals Private Limited, Bangalore, India and Merck (India) Limited, Mumbai, India.

2.3. Preparation and administration of I3C

Indole-3-Carbinol (I3C) was administered orally as a suspension using 5.5% propylene glycol in water. It was prepared each day just before treatment.

2.4. Median lethal dose (LD_{50}) determination of I3C

The oral LD_{50} dose of the compound I3C was carried out as per the instruction by Organization for Economic Co-operation and Development (OECD) guidelines 425 by Up-and-Down-Procedure (UDP) [25].

2.5. Experimental design for the toxicity study of the compound I3C

The animals were divided in to five groups containing six animals (n = 6) in each group.

- **Vehicle-control group (VC):** Each animal was orally treated with 5.5% propylene glycol in water for 28 days.
- **I3C (10 mg/kg b.w.)-treated group (I3C-10):** I3C was given orally at the dose of 10 mg/kg b.w. for 28 days.
- **I3C (20 mg/kg b.w.)-treated group (I3C-20):** I3C was given orally at the dose of 20 mg/kg b.w. for 28 days.
- **I3C (30 mg/kg b.w.)-treated group (I3C-30):** I3C was given orally at the dose of 30 mg/kg b.w. for 28 days.
- **I3C (40 mg/kg b.w.)-treated group (I3C-40):** I3C was given orally at the dose of 40 mg/kg b.w. for 28 days.

The mice were sacrificed 24 h after the last dose administration, on day 29.

2.6. Experimental groups for the evaluation of chemoprotective potential of I3C

In the present study, the animals were divided into five groups containing six animals (n = 6) in each group.

- **Vehicle control group (VC):** Each animal was given 5.5% propylene glycol in water by oral gavages for 10 days.
- **Only I3C treated group (I3C):** Each animal was orally treated with I3C at the dose of 20 mg/kg b.w. throughout the experimental period (25 days).
- **DOX treated group (DOX):** Each animal was intraperitoneally treated with only DOX in saline water (alternated days at a dose of 5 mg/kg b.w.) for up to day 9 (5 doses, resulting in a cumulative dose of 25 mg/kg b.w.).
- **DOX + I3C Concomitant-treated group (DOX + I3C Con):** I3C was treated orally at the dose of 20 mg/kg b.w. from day 1 to day 10 and DOX was administered as DOX-treated group.
- **DOX + I3C Pre-treated group (DOX + I3C Pre):** I3C was administered orally for 15 days prior to DOX treatment at the dose of 20 mg/kg b.w. and DOX was administrated as DOX treated group.

The mice were sacrificed on day 11 and the parameters described below were studied.

Doxorubicin dose was selected on the basis of (a) when DOX used in combination with other chemotherapy drugs for the treatment of different type of cancer, the most commonly used dosage of doxorubicin is 60–75 mg/m^2 IV once every 21 days. This dosage is equivalent to 20–25 mg/kg b.w. in mice (b) DOX in this clinically relevant dose is previously reported for its cardiotoxicity in mice [26,27]. On the basis of above back ground doxorubicin dose was selected at 25 mg/kg b.w. Dose of I3C was selected on the basis of some toxicity and antioxidant efficacy parameters following 28 days treatment. The results showed that I3C at 20 mg/kg b.w. is safe and most efficacious (Supplementary Table S1–S4). The organ sparing property of I3C was also confirmed by histological assessment (Supplementary Figure S1). So, on the basis of safety and efficacy end points, the oral dose of I3C at 20 mg/kg b.w. was selected for further preclinical study. In the present study we