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Cytogenotoxicological defense of retinyl palmitate in the front damage of antineoplastics

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ABSTRACT

Cancer, the multifactorial pathology and to date is the most lethal causes of death in the world. Cyclophosphamide (CPA) and doxorubicin (DOX) are the individually or combindly used two anticancer drugs. The antineoplastic drugs-mediated genetic instability can be overcome by using antioxidants. The study evaluated the cytogenotoxic modulatory potentials of retinyl palmitate (RP) caused by CPA and DOX in *Swiss* mice. For this, adult *Mus musculus* of either sex were divided equally regarding to the gender. Toxicogenetic effects were induced by the intraperitoneal (i.p.) administration of the CPA (20 mg/kg) and/or DOX (2 mg/kg), following to test for comet assay and micronucleus test in bone marrow cells after 48 h (DOX) and 7 h (CPA) of the administration of RP (100 IU/kg). Both CPA and DOX significantly ($p < 0.05$) increased with the index and frequency of damages, clastogenic and/or aneugenic effects with the augmenting of micronuclei, demonstrating the cytotoxicity interference on the ratio of normochromatic to polychromatic erythrocytes and bone marrow cells of mice, that were found to reduce in RP treatment groups. In conclusion, RP has a modulatory effect on CPA and DOX-mediated cytogenotoxic events. The findings may be a good indication to manage the antioneoplastic drug-induced stress mediated detrimental effects by using RP, especially as a side effect minimizer.

1. Introduction

Cancer is a collective term of more than 200 diseases characterized by an intense process of proliferative neoplastic cells. Treatment with the chemotherapeutic agents affects cellular processes of both normal and cancerous cells, thus the chance of secondary cancer (Hassan et al., 2016). Chemotherapeutic agents are used both individually or combindly (Ba-Sang et al., 2016).

Doxorubicin (DOX) is an antibiotic of the anthracyclines that acts by: merging the DNA molecule through the inhibition of the synthesis of DNA itself or their interaction with the TOP2A, topoisomerase II; and formation of free radicals that cause oxidative damage to the cell membranes, cellular other macromolecules such as carbohydrates, lipids and proteins as well as genetic materials. On the other hand, cyclophosphamide (CPA) is usually a prodrug, needing bioactivation by the liver for its anti-tumoral activity. It is a nitrogen mustard, considered as an alkylating agent and oxidizer. It inhibits DNA replication by promoting the alkylation of the nucleophilic groups, such as N7 guanine in DNA double strand (Emadi et al., 2009; Chennuru and Saleem, 2013).

Notably, the quickly renewable tissues, like bone marrow, suffer with the oxidative effects of chemotherapeutic agents, stimulating set up a more reliable laboratory protocol to investigate the risk associated with these kinds of agents (Brown, 2016).

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In order to alleviate the toxic effects of chemotherapy, nutritional supplementation consisting of antioxidants are thought to be a better choice in cancer. The retinyl palmitate (RP) ($C_{36}H_{60}O_2$) known as vitamin A palmiate is commonly used in cosmetic and aesthetic products to stimulate the production of elastin and collagen, which slows down the skin aging. It is also known for its powerful antioxidant effects in a number of test systems (NIH, 2012; Oliveira et al., 2009).

Cytogenetic biomarkers are important for the biomonitoring of damaging and toxic effects of a wide variety of substances. However, the direct research in humans is restricted and needs potential ethical approval. In this context, the non-clinical studies with experimental animals are the most effective tool in screening many chemicals/biologicals (Akyil and Konuk, 2015). Thus, this study aimed to assess the effects of RP on the cytogenotoxicological changes induced by CPA and/or DOX in mouse bone marrow through the application of comet and micronucleus tests.

2. Materials and methods

2.1. Sources of reagents and chemicals and preparation of samples

All the necessary chemicals and reagents, including CPA, DOX and RP were purchased from the Sigma-Aldrich (St. Louis, MO; USA). The CPA, DOX and RP were diluted in sterile saline solution 0.9% to attain the doses 20 mg/kg, 2 mg/kg and 1000 IU/kg, respectively.

2.2. Experimental animals and treatments

The study protocol was approved by the Ethics Committee of the Animal Experimentation of the Federal University of Piaui (UFPI), Teresina (PI), Brazil under the number: 081/14. A total 80 adult Swiss albino mice (*Mus musculus*) of either sex (2 months old), weighing between 25 and 30 g were collected from the Animal house Department of UFPI. The animals were kept in a controlled environment (temperature: 22 ± 1 °C with a 12 h dark-light cycle and freely access to food Purina®) and water *ad libitum*. In 8 groups, the animals were distributed in a manner that each contains equal number of male and females. The treatment period was set as per the half-life of the antineoplastic drugs. Table 1 shows the dose and treatment patterns of each group.

2.3. Comet assay in mouse bone marrow cells

Treatments (DOX, CPA and RP) were given intraperitoneally (i.p.). The animals were then euthanized by the administration of pentobarbital sodium (150 mg/kg, i.p.) and sacrificed. The comet assay was performed according to the method described by Uno et al. (2015). Briefly, after collecting the bone, 10 μL of the bone marrow sample was mixed with 90 μL of 0.75% w/v *low-mellting* agarose (melting point: 37 °C). The mixture was then spread on a pre-coated slide with normal melting point agarose (0.75% w/v) and covered with coverslips (24 × 60 mm). The slides were then kept at room temperature until solidification. After removal of coverslips, slides were then treated with lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100, 10% DMSO, pH 10) at 4 °C and protected from light, for 72 h. Electrophoresis was performed (electrophoresis medium: 300 mM 1 mM EDTA, NaOH, pH > 13) at 4 °C for 20 min at 25 V and 300 mA. Subsequently, the slides were treated with neutralizing solution (0.4 M Tris, pH 7.5) for 5 min; and exposed to the fixing solution (trichloroacetic acid 15%, zinc sulphate heptahydrate 5%, 5%) and glycerol for 10 min. After air drying, the slides were stained with silver nitrate solution (0.02%) and washed with distilled water by 3 times. Then the slides were dipped into stop solution (acetic acid) for 5 min, following to 3 times washing with distilled water and air drying at room temperature. Photomicrography was carried out at 40× by using an optical microscope to count 100 cells per slide (2 per animal) with the identification of 5 classes of damages: class 0 (genetic material without damage or intact); class 1, class 2, class 3 (low to medium damage) and class 4 (maximum damage), respectively. Finally, index of damage (ID) and frequency of damage (FD) were determined.

Table 1
Treatment scheme of the experimental animals.

Groups	Treatments	No of animals
Negative control	Without treatment	
	CPA (20 mg/kg)	
Positive control	DOX (2 mg/kg)	
	AC protocol (20/2 mg/kg)	n = 10 (male: 5; female: 5)
	RP (100 IU/kg)	
	CPA + RP	
Test groups	DOX + RP	
	AC + RP	

2.4. Micronucleus test in mouse bone marrow cells

The micronucleus test was carried out according to Morais et al. (2016). Briefly, bone marrow cell suspension (25 μL) was mixed with 0.3 mL of fetal bovine serum (20%) on the previously coded slides. Then, the slides were stained with Giemsa (10%) (Merck) in phosphate buffer 0.2 mol/L, pH 5.8. The counting of normochromatic erythrocytes (NCE), polychromatic erythrocytes (PCE) and micronuclei in polychromatic erythrocytes (MNPCE) were carried out by photomicrography at 1000× for 400 cells/treatment. The ratio of PCE/NCE was also determined.

2.5. Statistical analysis

Results were expressed as mean ± standard deviation (SD) (n = 10). Analysis of variance (ANOVA) followed by Bonferroni *post hoc* test was performed by using GraphPad Prism (version: 6.00) (GraphPad Software, San Diego California USA) considering $p < 0.05$.

3. Results and discussion

3.1. Antigenotoxic effects of RP

The alkaline comet assay is popularly used in the monitoring of a genotoxic potential wide variety of substances (Cash et al., 2014). In this study, the CPA and DOX as well as their combination treatment significantly ($p < 0.05$) induced genotoxic damages in mouse bone marrow cells (Table 2). Genotoxicity is a general term relates toxic impacts on the genetic materials such as DNA/RNA by the harmful endogenous or exogenous substances (Abbotts et al., 2014). The antitumor effects of chemotherapeutic drugs have the ability to cause damage to genetic materials by the action of free radicals, alkylation of the DNA molecule, inhibition of the activity of enzymes related to cell division, such as topoisomerases, inhibition of the repair machineries, thus results the cell death (Yard et al., 2016).

The AC group found to induce significant ($p < 0.05$) genetic damage ($p < 0.05$) when compared to the NC as well as CPA and DOX. RP alone was found to produce almost similar ID and FD as compared to the NC group. However, RP when co-treated with CPA and/or DOX, significantly ($p < 0.05$) reduced the ID and FD in animals. More reduction was observed in the AC + RP group then followed by CPA + RP and DOX + RP, respectively. However, RP