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Albendazole as a promising molecule for tumor control

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ABSTRACT

This work evaluated the antitumor effects of albendazole (ABZ) and its relationship with modulation of oxidative stress and induction of DNA damage. The present results showed that ABZ causes oxidative cleavage on calf-thymus DNA suggesting that this compound can break DNA. ABZ treatment decreased MCF-7 cell viability (EC_{50}=44.9 for 24 h) and inhibited MCF-7 colony formation (~67.5% at 5 μM). Intracellular ROS levels increased with ABZ treatment (~123%). The antioxidant NAC is able to revert the cytotoxic effects, ROS generation and loss of mitochondrial membrane potential of MCF-7 cells treated with ABZ. Ehrlich carcinoma growth was inhibited (~32%) and survival time was elongated (~50%) in animals treated with ABZ. Oxidative biomarkers (TBARS and protein carbonyl levels) and activity of antioxidant enzymes (CAT, SOD and GR) increased, and reduced glutathione (GSH) was depleted in animals treated with ABZ, indicating an oxidative stress condition, leading to a DNA damage causing phosphorylation of histone H2A variant, H2AX, and triggering apoptosis signaling, which was confirmed by increasing Bax/Bcl-xL rate, p53 and Bax expression. We propose that ABZ induces oxidative stress promoting DNA fragmentation and triggering apoptosis and inducing cell death, making this drug a promising leader molecule for development of new antitumor drugs.

1. Introduction

Cancer is a major public health problem in many parts of the world. In Brazil, about 57,000 of new cases of breast cancer were expected to be diagnosed in 2015 [1]. Standard cancer treatments are surgery, radiation, immunotherapy and/or chemotherapy. To the moment cancer management by chemotherapy is one of the most effective and potent strategies to treat malignant tumors. However, the multi-drug resistance, which is the mechanism that many cancer cells develop drug resistance to chemotherapy, has been a significant impediment to successful treatment [2]. On this context, in which increased cancer diagnosis and lack of an optimal treatment is observed, new therapeutic alternatives are required. On the other hand, drug repositioning is an important pharmaceutical strategy for drug development because it is faster and reduces drug risks once the safety and pharmacokinetic profiles of drugs are already well- known. Repositioning can offer a better risk versus-reward trade-off compared to other strategies for drug development. Furthermore, this strategy is economically attractive when compared to the cost of drug development based on de novo drug discovery and development [3].

Albendazole (methyl N-(6-propylsulfanyl-1H-benzimidazol-2-yl) carbamate; ABZ) is a benzimidazole derivative that was introduced in 1982 as an antihelmintic [4]. The mechanism of action of ABZ is related to microtubule inhibition and blocking glucose uptake that causes depletion of glycogen stores and lowering formation of ATP in the larval and adult stages of susceptible parasites. Altogether, it leads to immobilization and death of the parasite [5,6]. There are some few evidences in the literature supporting ABZ repositioning for cancer therapy. ABZ possesses anti- proliferative effects against several tumor cell lines [7–10]. Additional reasons to assess ABZ repositioning can be deduced from the fact that targeting the altered redox status of cancer cells is an interesting approach for new cancer therapy.

Cancer cells are under high oxidative stress because they possess altered antioxidant defenses and some researchers claim that reactive oxygen species (ROS) induced by anticancer drugs produce a shift in cellular antioxidant machinery as well as in the mitochondrial membrane potential, which are related to induction of programmed cell death [11]. It is important to stress out that ABZ showed increased ROS production, thereby promoting oxidative stress [12]. And this could be used as approach in cancer treatment considering cancer cells

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evading the apoptotic signaling [13]. In addition, due to the role of ROS as cellular switches for signaling cascades this property could be related with prooncogenic promotion or antitumorigenic signaling pathways, making the use of redox-modulating as an interesting strategy for anticancer therapy [14].

The aim of this work was to evaluate the antitumor effects of ABZ and relationship with induction of oxidative stress and DNA damage accessed by oxidative cleavage of CT-DNA, intracellular ROS content in MCF-7 cells and the related oxidative stress modulation, as well as the cycle cell arrest in Ehrlich ascites in mice treated with this drug, trying to characterize ABZ as a molecule prototype for possible drug repositioning in cancer therapy.

2. Material and methods

2.1. Chemicals and antibodies

Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS) and antibiotics were purchased from Gibco (USA). Albendazole (PubChem CID: 2082), methotrexate, calf thymus DNA (CT-DNA), agarose, DMSO, 2′,7′-dichlorofluorescein diacetate (DCFH-DA), N-acetyl-L-cysteine (NAC), 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB), bovine serum albumin (BSA), ethidium bromide (EtdBr), protease inhibitor cocktail, hydrogen peroxide, trichloroacetic acid, thiobarbituric acid, glutathione oxidized form (GSSG), NADPH and epinephrine purchased from Sigma Aldrich (Brazil). The phosphatase inhibitor cocktail was from Calbiochem (Merck Biosciences). Antibodies against γH2AX, p53, Bax and Bcl-xL were obtained from Santa Cruz Biotechnology, Inc. (USA). Mouse antibody against β-actin was from Millipore (USA). The secondary antibodies and the kit for chemiluminescence detection of HRP-coupled antibodies were from Millipore (USA). PI/RNAse solution kit from Immunostep (Salamanca, Spain).

2.2. Oxidative cleavage of CT-DNA

CT-DNA oxidative damage was evaluated using CT-DNA (0.5 mM) in 50 mM phosphate buffer (pH 7.2) incubated with 10 μM albendazole at 37 °C for 24 h. The samples were treated with 2-thiobarbituric acid 1% in 50 mM NaOH and glacial acetic acid, and incubated at 100 °C for 30 min. After cooling, absorbance was measured at 532 nm. The control had all components except albendazole. Fe(EDTA)-2: H_2O_2 was used for the positive control (100 μM/10 mM) [15].

2.3. Cytotoxicity, anti-proliferative assay, intracellular levels of ROS and mitochondrial membrane potential (ΔΨm) determination

Human breast cancer cell line MCF-7 was purchased from the Rio de Janeiro cell bank, Brazil. Cells were cultured in DMEM high glucose with FBS 10%, penicillin (100 U/mL) and streptomycin (100 μg/mL). MTT assay was used to study the effect of ABZ on cell viability [16]. Cells (10^4 cells/well) were placed on 96-well plates and after confluence they were exposed to ABZ (0–100 μM) for 24 h. Cells were washed and after 2 h of incubation with MTT (0.5 mg/mL), formazan crystals were solubilized in DMSO. The colored solutions were read at 550 nm. The results were presented as values of concentration EC_{50}.

Proliferation was evaluated *in vitro* through the colony forming unit assay. MCF-7 cells at density of single cells (500 cells) were allowed to set in six-well plates for 24 h. After, the medium was replaced by other containing of ABZ and MTX at non- cytotoxic concentrations (5 and 10 μM) and incubated for a further 24 h. In control wells, the cells were incubated in medium containing only DMSO 0.1%. After treatment, the cells were washed with warm PBS and fresh medium was provided. The cells were incubated for 16 days when the proliferation was counted in terms of colony forming units (CFUs) [17].

Intracellular ROS content were evaluated as reported by [18]. MCF-7 cells (15,000) were loaded with DCFH-DA (10 μM) in HBSS at 37 °C and incubated for 30 min. Excess DCFH-DA was removed by washing with fresh HBSS. After, the cells were incubated for 1 h with ABZ (5–25 μM) and methotrexate (MTX; at same concentrations), washed twice with HBSS, and then 100 μl of HBSS/well was added. The intensity of fluorescence was measured at 485 nm for excitation and 530 nm for emission using a Multiscan microplate reader.

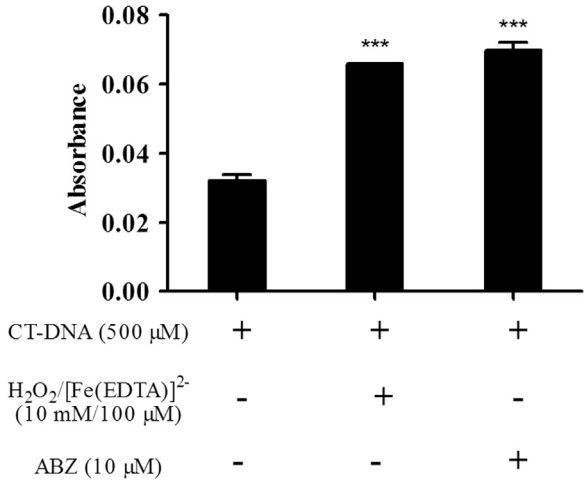

Fig. 1. Evaluation of CT-DNA cleavage in presence of albendazole (ABZ). The analysis was assessed by absorbance of thiobarbituric reactive species (TBARS) in CT-DNA (500 μM) treated with ABZ (10 μM). Negative control (NC): phosphate buffer. Positive control (PC): [Fe(EDTA)]$^{2-}$/H_2O_2. Data represent the mean of three independent experiments. Significant at ***$p < 0.001$ compared to the control group.

Mitochondrial membrane potential was performed using a fluorescent probe TMRE. MCF-7 cells (10^4/well) were plated in fluorescence 96-well plate, after confluence the cells were treated with different concentrations of ABZ (1, 10 and 100 μM), NAC (5 mM) or ABZ associated with NAC. After 6 h of treatment the cells were washed once with HBSS and incubated with TMRE (1 μM) during 20 min at 37 °C. After the cells were washed once with HBSS, followed by fluorescence intensity measurement, using excitation peak of 549 nm and emission of 575 nm.

2.4. Ehrlich carcinoma growth inhibition in mice

The antitumor effects of ABZ were evaluated against the Ehrlich ascitic carcinoma inoculated into the abdomen of isogenic Balb-c mice (20 ± 2 g). Animal procedures were conducted in accordance with legal requirements and the approval of the local ethics committee (CEUA/UFSC PP00784) and legal requirements (NIH publication #80-23, revised in 1978). Animals were housed under controlled conditions and had free access to laboratory food and water.

Tumor induction was carried out by inoculating 5×10^6 cells of Ehrlich carcinoma. Twenty-four hours later mice were divided into 3 groups (n=12): The control was treated with saline (50 μl). The test-group was treated with ABZ 20 mg/kg in the same volume of vehicle (50 μl). MTX (2.5 mg/kg) was used for the positive control [19]. The dose of ABZ was chosen previously considering the maximum saturation point of this drug. The treatment started 24 h after tumor inoculation and the abdominal circumference of all animals was measured (time zero). It was repeated every 24 h during nine days. On the tenth day, the abdominal circumference of all animals was measured. Then, half of each group was euthanized for the evaluation of the ascitic fluid. Tumor growth was determined using the following equation [20]: Inhibition of tumor growth (%)=100- [(variation in waist circumference of the treated group×100)/variation in waist circumference of the control group]. Mice (n=6) from each group were kept alive to determine survival time [21,22].