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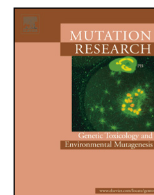

4-Aminoantipyrine reduces toxic and genotoxic effects of doxorubicin, cisplatin, and cyclophosphamide in male mice

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ABSTRACT

The analgesic drug dipyrone is used to treat side effects (including pain and fever) of cancer chemotherapeutic agents. Dipyrone is metabolized to 4-aminoantipyrine (4-AA), a PGE2-dependent blocker and inhibitor of cyclooxygenase (COX). We evaluated the genotoxic, mutagenic, apoptotic, and immunomodulatory activities of 4-AA *in vivo* and the effects of its combination with the antineoplastic drugs doxorubicin, cisplatin, and cyclophosphamide. 4-AA did not cause genotoxic/mutagenic damage, splenic phagocytosis, or leukocyte alterations. However, when combined with the antineoplastic agents, 4-AA decreased their genotoxic, mutagenic, apoptotic, and phagocytic effects. These results suggest that 4-AA might interfere with DNA damage-mediated chemotherapy.

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1. Introduction

The accumulation of mutations is associated with the uncontrolled proliferation of tumor cells [2]. Most cancer chemotherapeutic agents cause DNA damage, leading to cell cycle arrest and programmed cell death. However, therapy is hampered by the limited specificity of most chemotherapies [1,2]. Doxorubicin (Dox), cisplatin (cisplat), and cyclophosphamide (CP) are commonly used damage-inducing chemotherapy [3–6]. Dox causes double-stranded DNA breaks via topoisomerase II inhibition [3]. Both cisplat and CP are alkylating agents that cause interstrand crosslinking and inhibition of DNA synthesis [3,6]. Unlike CP, however, cisplat does not require hepatic metabolism and it causes intrastrand as well as interstrand crosslinking [4,5].

Most chemotherapeutic drugs also damage the DNA of normal cells and cause side effects (including pain and fever) which may be treated with analgesics such as dipyrone [7,8]. This drug is not available in USA, UK, or Japan, but it is still marketed without prescription in many countries, including most South American countries, France, Switzerland, Russia, India, etc. [9,10]. Intestinal hydrolysis of dipyrone forms N-methyl-4-aminoantipyrine (MAA), which is absorbed and then metabolized in the liver, resulting in 4-aminoantipyrine (4-AA), which can then be acetylated. These metabolites inhibit prostaglandin synthesis via inhibition of cyclooxygenases (COX), which blocks the transduction of pain signals and consequently relieves pain and fever [10,11]. MAA can cause hypothermia [12], but 4-AA is a PGE2-dependent blocker that can reduce fever without inducing hypothermia. These observations suggest that MAA has a more complex spectrum of biological activities than 4-AA.

Despite the benefits of dipyrone, little is known about possible interactions between its metabolites and chemotherapeutic agents. Here, we have studied the genotoxic, mutagenic, apoptotic, and immunomodulatory effects of 4-AA and its effects in combination with Dox, cisplat, and CP.

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2. Materials and Methods

2.1. Chemicals and study design

4-Aminoantipyrine (4-AA; CAS-83-07-8; Sigma-Aldrich, St. Louis, MO, USA) was prepared in dimethyl sulfoxide (DMSO) and then diluted in saline solution with 5% glucose (final concentration of DMSO, 5%). The solution was administered by intraperitoneal (i.p.) injection of 12 or 24 mg/kg body weight (b.w.). 4-AA doses were based on literature and selected after a pilot study in mice to confirm lack of toxicity [13,14]. The saline/DMSO (5%) vehicle solution was administered i.p. to all groups, including negative and positive controls, at final volume 0.1 ml/10 g b.w.

The chemotherapeutic agents were administered in single i.p. doses previously defined in pilot studies (data not shown). Dox (Glenmark Farmacêutica Ltda, N° REG MS 1.1013.0232.002-4, Brazil) was diluted in distilled water and administered at 16 mg/kg b.w. Cisplatin (ACCORD Farmacêutica, N° REG MS 1.5537.0002.003-7, Brazil) was diluted in distilled water and administered at 6 mg/kg b.w. Cyclophosphamide (Genuxal, Baxter Hospitalar Ltda, N° REG MS 1.00683.0168.003-1, Brazil) was diluted in distilled water and administered at 100 mg/kg b.w.

Sixty male adult (8-10 weeks) mice *(Mus musculus)*, average weight 35 g, were obtained from the State Agency for Animal and Plant Sanitary Vigilance. Mice were maintained in polypropylene boxes covered with wood shavings and placed in ventilated ALESCO® cabinets. Mice were kept under controlled climate and light conditions (12 h light/12 h dark; 22 ± 2 °C; relative humidity 55 ± 10%), fed with commercial feed (Nuvital, Nuvilab®), and provided filtered water *ad lib.* The study was conducted according to the norms of the Brazilian College of Animal Experimentation, was compliant with the directions set by the Universal Declaration of Animal Rights, and met with the approval of the Federal University of Mato Grosso do Sul Ethics Committee on Animal Use (Protocol n° 399/2012).

The animals were divided into two groups. In the first group, compounds were administered separately. In the second group, the chemotherapeutic agents were combined with 4-AA. Each group was divided into six lots with five animals each (Fig. 1). After the experiments, the animals were euthanized by cervical dislocation for the harvesting of biological materials.

2.2. Biological assays

2.2.1. Comet assay

Peripheral blood was collected 24 h after treatment and used for the comet assay according to Singh [15] and modified by Carvalho [16]. 100 cells per animal were analyzed with a fluorescence microscope (BIOVAL, Model L2000A, São Carlos, São Paulo – Brazil) at 40 x magnification with excitation filter 420–490 nm and 520 nm barrier filter. Cells were classified into four classes (0, 1, 2 and 3) indicating increased comet length relative to the nucleus. The scores were calculated by multiplying the comet class by the number of damaged cells for each class.

2.2.2. Micronucleus assay

The micronucleus assay was performed according to the protocol described by Hayashi [15] and modified by Carvalho [16]. Peripheral blood, approx. 20 μL, was collected 24, 48, and 72 h after treatment. Samples were deposited on a slide previously coated with acridine orange (20 μL, 1 mg/ mL). The slides were kept in the freezer (−20 °C) for at least 2 weeks and subsequently analyzed for 2,000 cells per animal with the fluorescence microscope, as above.

2.2.3. Apoptosis assay

Animals were euthanized 72 h after treatment, followed by laparotomy to collect the liver and kidneys. The material was processed according to Navarro [17]. To prepare the slides, was used samples of macerated liver or kidney tissue, 100 μL. Slides were fixed in Carnoy's solution for 5 min and subjected to decreasing concentrations of ethanol (95%, 75%, 50%, 25%). Subsequently, slides were washed in McIlvaine buffer for 5 min, stained with acridine orange (0.01%, 5 min), and washed again with buffer. One hundred cells per animal were analyzed identification of apoptotic cells was carried out through analyses of patterns of DNA fragmentation according to Carvalho [16] and Navarro [17].

2.2.4. Phagocytosis assay

Animals were euthanized 72 h after treatment, followed by laparotomy to collect the spleen. Spleens were macerated in saline solution and cell suspension (100 μl) was deposited on a slide previously stained with blood orange acridine (1 mg/ml), and then coated with a coverglass. The analysis was performed with the fluorescence microscope, as above. For this test, 200 cells per animal were analyzed, then classified based on the presence or absence of phagocytosis according to the protocol of Carvalho [16].

2.2.5. Differential blood cell count

Peripheral blood was collected 72 h after treatment to produce each slide. One hundred cells per animal (categorized into lymphocytes, neutrophils, monocytes, eosinophils, and basophils) were analyzed [17,18].

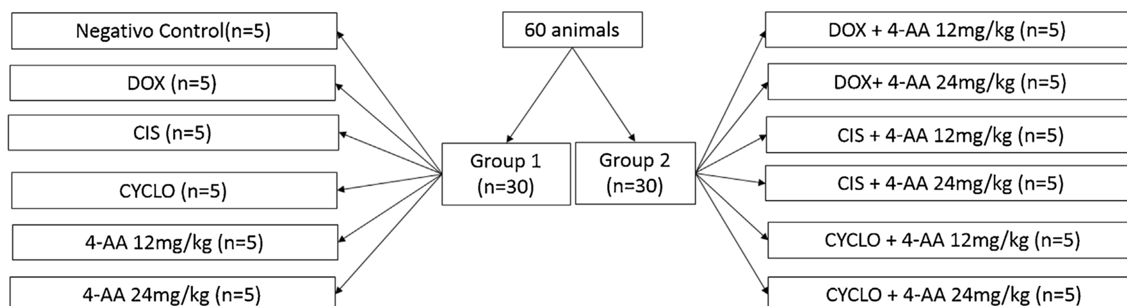

Fig. 1. Experimental design. Sixty animals were divided into two batches; each batch was subdivided into six groups of five animals each. The groups on the left side were treated with chemotherapeutic drugs alone. On the right side are the groups treated with chemotherapeutic drugs plus 4-AA. The saline/DMSO (5%) vehicle solution was administered to all groups, including negative and positive controls, at 0.1 ml per 10 g b.w.

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