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Protective effect of Spirulina against cyclophosphamide-induced urotoxicity in mice

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ABSTRACT

Cyclophosphamide (CP) is an anti-neoplastic drug, which is widely used for treating cancer and non-malignant tumors. One of the major side effects of CP is hemorrhagic cystitis. Spirulina (*Arthrospira platensis*; Sp) is a blue-green algae with the ability to attenuate oxidative stress, which may be utilized for alleviating side effects of chemotherapeutic drugs in the clinic. The aim of the present study was to evaluate the ability of *Sp*, to protect mice from cyclophosphamide-induced urotoxicity and hemorrhagic cystitis due to its antioxidant properties. Adult female mice were orally administered Sp (600 g/kg body weight/day) over nine days as well as a single dose of CP (40 mg/kg body weight) intraperitoneally either four days previously (CP + Sp group) or four days after the start of Sp intake (Sp + CP group); two further groups were treated with either Sp or CP only, respectively. The results showed that CP induced hemorrhagic cystitis in mice, with levels of malondialdehyde (MAD) significantly increased and those of glutathione (GSH) decreased compared with the control group ($P < 0.05$), while the opposite effects were observed in the mice who received Sp only ($P < 0.05$). Furthermore, in the CP + Sp group, MAD and GSH levels were improved compared with those in the CP only group, and in the Sp + CP group, the effects of CP were reversed. In addition, histomorphological alterations of the urinary bladder were significantly lower than those in the CP group. In conclusion, pre-treatment with Sp protected mice from CP-induced urotoxicity, probably via its anti-oxidant and anti-apoptotic properties.

Introduction

CyclophoSphamide (CP) has been used in the treatment of certain types of cancer (lymphoma, leukemia and multiple myeloma) and non-malignant diseases such as rheumatoid arthritis [1]. However, CP has numerous adverse effects, a major one being hemorrhagic cystitis (HC), which arises from acute inflammation of the urinary bladder [2,3]. Acrolein, a metabolic product of CP, rapidly reacts at numerous cellular sites and depletes cellular antioxidants [4]. It can also react with certain protein residues and nucleophilic sites of the DNA [5].

In addition, CP has been identified as an initiator of lipid peroxidation [6]. Acrolein, the metabolic product of CP, is a reactive and relatively electrophilic compound, and its high toxicity is associated with its ability to rapidly enter the uroepithelium due to its chemical nature as an un-saturated aldehyde as well as to increase the production of reactive oxygen Species (ROS) in the bladder epithelium [7]. Numerous studies have used antioxidants, including α-tocopherol, β-carotene and melatonin [8,9], as protective agents against CP-induced bladder damage with the elimination of the urotoxicity of CP improving the tolerability of the drug [1].

Spirulina (Sp; *ArthroSpira platensis*) is a photosynthetic cyanobacterium that possesses biological activity and is widely cultivated for the production of nutritional supplements [10]. Sp has also gained increasing attention from medical scientists as a nutraceutical and source of potential pharmaceuticals. Indeed, numerous active components of Sp, including phycocyanins, tocopherols, β-carotenes and phenolic compounds exhibit antioxidant properties [11]. Sp is rich in fatty acids (linoleic acid, F-linolenic acid and palmitic acid), essential amino acids, selenium (Se) and vitamins A–E [12].

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Malondialdehyde (MDA) is formed as a product of free oxygen radicals during oxidative degeneration of lipids [13] and serves as an indicator of oxidative stress [14]. Studies have shown that ROS have an important role in CP-induced HC [3,2].

Biological systems possess numerous biomolecular agents to protect themselves from damage through free radicals. Glutathione (GSH) is the most abundant intracellular thiol-based anti-oxidant and has an important role in the cellular defense cascade against oxidative injury [15]. GSH, a cofactor for glutathione peroxidase, catalyzes the reduction of hydrogen peroxide to oxygen and water, thereby limiting the formation of hydroxyl radicals, which are highly toxic ROS [16].

The activities of enzyme as antioxidant are dependent on certain metalloenzymes, which in turn require trace elements, micro-nutrients present at very low concentrations (μg/dl) in bodily fluids, to function effectively [17]. Zinc (Zn) and selenium (Se) are examples of trace elements that we include them in our study.

The aim of the present study was to demonstrate, biochemically as well as histopathologically, the toxic effects of CP on the bladder and to assess the capacity of Sp extract to alleviate this side effect.

Materials and methods

Animals

A total of 50 female BALB/C mice (age, 6 weeks; weight, 25–30 g) were obtained from the Animal Resources Unit of the Medical Experimental Research Center (MERC), Faculty of Medicine, Mansoura University (Mansoura, Egypt). The mice were kept under a 12-h light/dark cycle in clean plastic cages with free access to food (standard mouse pellet diet) and water *ad libitum*. The humidity was kept at 55 ± 10% and the temperature was controlled at 23 ± 1 °C.

All experiments using animals were performed according to the protocol approved by the Intuitional Animal Care and Use Committee at Mansoura University.

Drugs and agents

CP (Endoxa) was purchased from Baxter Oncology Chemical Co. (Egypt) and dissolved in 0.9% NaCl solution to prepare a stock solution, which was intraperitoneally (i.p.) injected into the mice. Sp was provided as a fine dark blue-green powder by the Botany Department Faculty of Science Zagazig University (Zagazig, Egypt) and was dissolved in sterile distilled water to prepare a stock solution which was orally administered to mice using intragastric gavage.

Experimental design

Mice were allocated to five groups (10 mice in each) as follows:

Group 1	The negative control (C) group	animals received one i.p. injection of 0.5 ml saline.
Group 2	The positive control (CP) group	mice received an i.p. injection of CP dissolved in 0.5 ml saline at a dose of 40 mg/kg body weight.
Group 3	Sp group	mice received an oral dose of Sp of 600 mg/kg body weight in 0.5 ml water for nine successive days.
Group 4	treatment group (CP + Sp)	mice were treated as in the CP group, and from day four after CP injection, mice were administered Sp at a dose of 600 mg/kg body weight.
Group 5	prophylactic group (Sp + CP)	mice were treated as in the Sp group for 9 days and were given one i.p. injection of CP (40 mg/kg body weight) on day four after the first dose of Sp.

All mice were sacrificed at the end of the 9 days. Blood samples of mice from each group were collected by cardiac puncture on the day of termination. Whole blood was withdrawn from each mouse, 2 ml of which were mixed with ethylenediaminetetraacetic acid for complete blood count using automatic hematology analyzer (Sysmex Corp., Kobe, Japan), and the remaining blood was left to stand in a plain tube for preparation of serum from clotted blood by centrifugation at 2000 rpm for 5 min. Serum was divided into two aliquots and immediately frozen at −70 °C for later assessment of MDA and GSH by ELISA (Calbiotech, Spring Valley, CA, USA; R&D Systems, Minneapolis, MN, USA) and Zn and Se were estimated using atomic absorption Spectroscopy [18].

Histological analysis

Following fixing in 10% formalin and embedding in paraffin, tissues were cut into 5-μm sections, mounted on slides and stained with hematoxylin-eosin (H-E) to visualize the general structure of the urinary bladder. The severity of bladder damage was assessed depending on the following phenomena: Edema, hemorrhage, desquamation of epithelial cells and destruction of smooth muscle cells.

Statistical analysis

Differences within groups were analyzed by one-way analysis of variance with SPSS software (version 10; SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered to indicate a statistically significant difference. Mean ± standard deviation values were calculated for each group to determine the significance of inter-group differences.

Results

Sp inhibits CP-induced oxidative stress in mice

At the end of the experiment (day 9), the serum MDA levels in the CP group were significantly higher than those in the control group, while those in the Sp group were significantly lower than those in the CP group ($P < 0.05$). Furthermore, serum GSH levels in the Sp group were significantly elevated, while those in the CP group were significantly reduced compared with those in the control group ($P < 0.05$) (Table 1).

Sp attenuates Cp-induced reduction of Zn and Se levels in mice

Administration of CP alone caused a significant drop in the serum levels of Zn and Se of the mice ($P < 0.05$). By contrast, treatment with Sp alone significantly elevated the serum levels of Zn and Se (Table 1). Ingestion of Sp (600 mg/kg) after CP significantly improved the lowered levels of Zn and Se compared with those in the CP group; however, they remained below their correSponding control levels. Administration of Sp prior to CP caused further

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