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Protective effects of boron on cyclophosphamide induced lipid peroxidation and genotoxicity in rats

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HIGHLIGHTS

- B reduces cyclophosphamide (CYC) induced toxicity.
- B inhibits lipid peroxidation and DNA damage in rats.
- B regenerates CYC-induced histopathological changes in rat tissues.
- B attenuates NO and restores SOD, CAT, and GSH in rats.

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ABSTRACT

The aim of the present study was to evaluate the possible protective effect of boron (B) on cyclophosphamide (CYC) induced oxidative stress in rats. Totally, thirty Wistar albino male rats were fed standard rodent diet and divided into 5 equal groups: physiological saline was given intraperitoneally (i.p.) to the control group (vehicle treated), to the second group only 75 mg kg^{-1} CYC was given i.p. on the 14th d, and boron was administered (5, 10, and 20 mg kg^{-1}, i.p.) to the other groups for 14 d and CYC (75 mg kg^{-1}, i.p.) on the 14th d. CYC caused increase of malondialdehyde and decrease of glutathione levels, decrease of superoxide dismutase activities in erythrocyte and tissues, decrease of erythrocyte, heart, lung, and brain catalase, and plasma antioxidant activities. Also, CYC treatment caused to DNA damage in mononuclear leukocytes. Moreover, B exhibited protective action against the CYC-induced histopathological changes in tissues. However, treatment of B decreased severity of CYC-induced lipid peroxidation and genotoxicity on tissues. In conclusion, B has ameliorative effects against CYC-induced lipid peroxidation and genotoxicity by enhancing antioxidant defence mechanism in rat.

1. Introduction

Cyclophosphamide (N,N-Bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine 2-oxide; CYC), is a cytotoxic bifunctional alkylating agent, belongs to the class of nitrogen mustards (Dollery, 1999; Tripathi and Jena, 2008). CYC is widely used at high dose for chemotherapy of various forms of cancer (bronchial, breast and ovarian cancer, lymphomas, leukemias, etc.) and at low dose for treatment of autoimmune diseases (rheumatoid arthritis), and also used as immunosuppressant after organ transplantations (bone marrow transplantations) (Jolivet et al., 1983; Kuo et al., 2003; Buerge et al., 2006). In clinical application, it is important to prevent DNA damage of normal cells which is induced by CYC (Tripathi and Jena, 2009). Recent studies suggest that CYC generates reactive oxygen species like superoxide anion, hydroxyl radical and hydrogen peroxide (H_2O_2) during its oxidative metabolism, and depresses the antioxidant defense mechanisms in the liver (Bhattacharya et al., 2003; Stankiewicz et al., 2002). These free radicals may attack soluble cell compounds as well as membranes, eventually leading to the impairment of cell functions and cytolysis (Bergendi et al., 1999).

Boron (B) is a mineral substance found in nature. A side from its traditional use in the health care system, B is widely used in

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industrial, agricultural, and cosmetic applications. It is absorbed rapidly after its administration and rapidly distributed throughout the body via passive diffusion. Following administration of B, the concentration of it in the blood and tissue is reported to be in the ratio of 1:1 in rats and humans (Murray, 1998). B is primarily essential for plants and some animals, and beneficial for humans in nutritional amounts, and thus found in animal and human tissues at low concentrations (Hunt, 1996). B deficiency may occur in animals when their diet contains B at 0.3 mg kg^{-1}; the maximum tolerable level of B is 150 mg kg^{-1} diet (Nielsen et al., 1987). B has effects on the metabolism of calcium and potassium (Meacham et al., 1994), vitamin D (Hunt, 1996), aldehyde dehydrogenase, xanthine oxidase, cytochrome B_5 reductase (Hunt, 1996; Devirian and Volpe, 2003), insulin, oestrogen, testosterone, T_3, T_4 (Nielsen et al., 1987; Armstrong et al., 2001), triglycerides, glucose (Hunt, 1996), and reactive oxygen species (Turkez et al., 2007; Ince et al., 2010). The most important application of B is neutron capture therapy; it has been used extensively in the context of various cancers (Martin et al., 1989; Gregoire et al., 1993; Primus et al., 1996).

The present study was designed to investigate the effect of B on lipid peroxidation (LPO), antioxidant status, DNA damage, and histopathological changes caused by CYC in Wistar albino rats.

2. Materials and methods

2.1. Materials

Boric acid as a source of boron and CYC purchased from Sigma–Aldrich (Interlab, Turkey), and Baxter (Halle, Germany), respectively. All the other chemicals and reagents were of analytical reagent grade purchased from commercial sources.

2.2. Experimental protocol

Healthy male Wistar albino rats, 60 d of age and weighing 250–300 g, were purchased from The Animal Breeding Laboratories of the Experimental Animal Research and Application Center (Afyon, Turkey). The animals were kept at room temperature (25 °C) and relative humidity (50–55%) in a 12 h light/dark cycle with *ad libitum* access to standard rodent diet and water. Rats were allowed to acclimatise to the animal facility for at least 7 d before experiment started. Prior to the experiments, rats were fed with standard rodent diet for one week in order to adapt to the laboratory conditions.

In this study, totally 30 male rats were randomly allocated into 5 groups, 6 rats in each group. All animals were fasted overnight before the experiment. Physiological saline was given intraperitoneally (i.p.) to the control group (vehicle treated), 75 mg kg^{-1} CYC was given i.p. alone (Oboh et al., 2011) to the second group on the 14th d, and B (5, 10, and 20 mg kg^{-1}, i.p.) was given to the other groups for 14 d and they received CYC (75 mg kg^{-1}, i.p.) on the 14th day. The experimental protocols were also approved by the Animal Care and Use Committee at Afyon Kocatepe University and were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.3. Blood collection and preparation of erythrocytes and homogenate

Blood samples from each group were collected by cardiac puncture into heparinised and non-heparinised tubes under light ether anaesthesia at the end of 14 d. Within 30 min of blood collection, the erythrocytes were precipitated by centrifugation at 3500 rpm for 15 min at 4 °C, afterwards plasma and serum were removed. The erythrocytes were washed three times with isotonic saline and the puffy coat was discarded. Then, same volume of isotonic saline and erythrocyte were added into vials and stored at −20 °C in the deep freeze. Erythrocyte suspension was destroyed by osmotic pressure, use five times of cold deionised water. The erythrocyte lysate was stored at 4 °C until measurements within 3 d (Winterbourn et al., 1975). Animals were sacrificed by cervical dislocation and their liver, lung, kidney, heart, and brain tissues were washed immediately with ice cold 0.9% NaCl. Each tissue was trimmed free of extraneous tissue, rinsed in chilled 0.15 M Tris–HCl buffer (pH 7.4). These tissues were blotted dry, and homogenised in 0.15 M Tris–HCl buffer (pH 7.4) to yield a 10% (w/v) homogenate. Then, they were centrifuged at 3500 rpm for 10 min at 4 °C. The pellets represented the nuclear fraction and the supernatants were subjected to centrifugation at 20000 rpm for 20 min at 4 °C. The resultant pellets and the supernatants represented the mitochondrial fraction and the cytosolic (including microsomal fraction) fraction, respectively. Reactive oxygen species generation was observed in these fractions as well as whole homogenate (Kucukkurt et al., 2008).

2.4. Preparation of tissues for histopathological analysis

At the end of experimental period, 30 male rats were sacrificed. Then, they were dissected and liver, lung, kidney, heart, and brain tissues from each animal were collected and fixed into 10% formalin solution for 48 h and then dehydrated through graded alcohol series (70–100%), cleared in xylene and embedded in paraffin. 5–6 μm thick paraffin sections were cut and stained with haematoxylin-eosin (H&E) and analyzed under a light microscope (Olympus Bx51 model, Tokyo, Japan) equipped with camera (Olympus DP20, Tokyo, Japan).

2.5. Measurement of LPO and reduced glutathione (GSH) in whole blood and tissue homogenates

Malondialdehyde (MDA), as a marker for LPO, was determined according to the methods of Draper and Hardley (1990) in whole blood and in tissue homogenates (Ohkawa et al., 1979). The principle of the methods is based on spectrophotometric measurement of the colour production during the reaction of thiobarbituric acid (TBA) with MDA and its absorbance was measured spectrophotometrically at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of MDA-TBA complex and expressed as μM in blood and nM in wet tissue.

GSH concentration was measured using the method described by Beutler et al. (1993) in whole blood and tissue homogenates. Briefly, 0.2 mL sample was added to 1.8 mL distilled water. 3 mL of precipitating solution (1.67 g metaphosphoric acid, 0.2 g EDTA and 30 g NaCl in 100 mL distilled water) was mixed with sample. The mixture was allowed to stand for approximately 5 min and then filtered (Whatman No. 42). 2 mL of filtrate was taken then it was added into another tube and 8 mL of the phosphate solution (0.3 M disodium hydrogen phosphate) and 1 mL 5,5′-Dithio-bis(2-nitrobenzoic acid) (DTNB) were added. A blank was prepared with 8 mL of phosphate solution; 2 mL diluted precipitating solution and 1 mL DTNB reagent. A standard solution of the GSH was prepared (40 mg/100 mL). The optical density was measured at 412 nm on the spectrophotometer. Results were expressed as μM in blood and nM in wet tissue.

2.6. Measurement of superoxide dismutase (SOD) and catalase (CAT) activities in erythrocyte lysate and tissue homogenates

The antioxidant enzyme activity of SOD in erythrocyte lysate and tissue homogenate was measured according to the method of Sun et al. (1988). The measurement of SOD is based on the

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