The Protective Effects of Spirulina in Cyclophosphamide Induced Nephrotoxicity and Urotoxicity in Rats

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OBJECTIVE To evaluate the role of *Spirulina*, a blue-green algae with antioxidant properties in the protection of cyclophosphamide-induced nephrotoxicity and hemorrhagic cystitis in rats.

METHODS The control group (C) was sacrificed 24 hours after being given a single dose of saline intraperitoneally (150 mg/kg) on the seventh day of the experiment. The rats in the second group (CP) were sacrificed 24 hours after being given a single dose of cyclophosphamide, intraperitoneally (150 mg/kg) on the seventh day of the experiment. *Spirulina* was administered to the third group (SP+CP) orally (1000 mg/kg bw/day) for 7 days and a single dose of cyclophosphamide was injected intraperitoneally (150 mg/kg) on the seventh day of the experiment. At the eighth day of the experiment, malondialdehyde, superoxide dismutase, and catalase levels in renal and urinary bladder tissues were measured. Histomorphology in urinary bladder, apoptosis by caspase 3 immunostaining, and TUNEL assay in kidney were also evaluated.

RESULTS Tissue levels of malondialdehyde in the SP+CP group were significantly lower versus CP group ($P < .05$). Tissue levels of superoxide dismutase and catalase in the SP+CP group were significantly higher vs the CP group ($P < .05$). The histomorphologic alteration in urinary bladder in the SP+CP group was significantly lower vs that in the CP group. In the kidney, apoptosis in the SP+CP group as shown with TUNEL assay and immunohistochemistry was significantly lower vs that in the CP group ($P < .05$).

CONCLUSION Pretreatment with *Spirulina* protects the rats from cyclophosphamide-induced nephro-urotoxicity via its antioxidant and anti-apoptotic properties. UROLOGY 80: 1392.e1–1392.e6, 2012. © 2012 Elsevier Inc.

Cyclophosphamide (Cyc) has been in clinical use since the late 1950s and is proved to be effective in the treatment of both neoplastic diseases, such as solid tumors and lymphomas, and nonneoplastic diseases, such as rheumatoid arthritis and systemic lupus erythematosus.[1] It is well known that this drug or its metabolites causes acute inflammation of the urinary bladder and may cause renal damage.[1] The damage is related to the Cyc itself and to its structural analogue ifosfamide, both of which are highly alkylating cytostatic compounds. Both are known to have severe urologic adverse effects, but the effects of Cyc on the kidney is controversial.[1-3] Cyc causes histopathologic changes in rat kidney, even if the plasma creatinine level, a reliable indicator of the glomerular function is not altered.[4] The renal damage in rats is recognized histopathologically as glomerular inflammation, epithelial cytoplasmic vacuolization in cortical tubules, interstitial edema, and mild hemorrhagic changes in the renal cortex and also biochemically, as reduced renal glutathione level and increased renal malondialdehyde (MDA) level 24 hours after Cyc treatment.[4] In children and elderly patients, Cyc treatment can also result in glomerular and tubular dysfunction because of its toxic effects on immature kidneys and because of the progressive decline in renal function with aging, respectively.[2] However, in most of the adult patients on Cyc treatment, the adverse effects of the drug or its metabolites in the kidney are usually neglected because of unchanged kidney function. As mentioned before, severe urinary bladder toxicity is also one of the most important adverse effects of Cyc, being the major limiting factor in its use. Clinically, this toxicity varies from transient irritative voiding symptoms and mild hematuria to life-threatening hemorrhagic cystitis.[5] The urotoxicity is related to the formation of toxic

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metabolites of Cyc in the liver and partly to the direct alkylating activity of the drug metabolites on the urinary tract.[5] In the liver, the cytochrome p-450 monooxygenase system converts Cyc to 4-hydroxy-cyclophosphamide and its tautomer aldophosphamide. Aldophosphamide undergoes β elimination to release acrolein and the alkylating compound phosphoramide mustard.[5] The mechanism of acrolein toxicity is that it binds to and causes the depletion of the cellular antioxidant nucleophiles, such as glutathione and it initiates the lipid peroxidation that results in hemorrhagic cystitis.[6] Many cytokines, such as tumor necrosis factor, interleukins, and transcription factors, also play a role in the pathogenesis of this inflammatory process.[5,7] By contrast, superoxide dismutase (SOD) and catalase are 2 powerful antioxidant enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide and the hydrogen peroxide to water and oxygen, respectively. Because of the plasma and the tissue, concentrations of various antioxidants were experimentally shown to be decreased during Cyc therapy.[2] The application of plant extracts that contain antioxidants to scavenge the harmful effects of Cyc attracted worldwide interest.[8,9] Among these, a blue-green algae, *Spirulina*, has been demonstrated to have antioxidant effects in many in vitro and in vivo studies.[10] It has a complex structure that contains mainly proteins, lipids, carbohydrates, vitamins, and a pigmented protein, C-phycocyanin. It has been used as a nutritional supplement and also for many therapeutic purposes.[10,11] Meanwhile, its nephroprotective benefits were also reported.[12,13] Its possible uroprotective effects have not yet been studied.

The present study was designed to show the adverse effects of Cyc on kidney and also to show the possible protective role of *Spirulina* against Cyc-induced nephrotoxicity and hemorrhagic cystitis in rats. To the best of our knowledge, the effects of *Spirulina* on Cyc-induced nephrotoxicity and urotoxicity in rats have not been reported in the English literature.

MATERIAL AND METHODS

A fine, dark blue–green powder of Hawaiian *Spirulina-Arthrospira platensis pacifica* (Algbiotek, Istanbul, Turkey) was dissolved in sterile distilled water and given orally. Cyc was purchased from Eczacibaşı/Baxter Chemical, Co. (Istanbul, Turkey). The study was approved by the Local Ethics Committee for Animal Experiments of Maltepe University Medical School, Istanbul, Turkey (Project# 2011/01). The dosage and the route of administration of Cyc were determined from that described in the literature.[14]

Animals and Treatment

Eighteen adult female Wistar albino rats (180-210 g) purchased from the Experimental Animal Laboratory of Maltepe University School of Medicine were used. The rats were housed under conditions of controlled temperature in individual cages in a room with a daily 12-hour light-dark cycle. Food and water were available ad libitum. The rats were divided randomly into 3 groups of 6 rats each. After acclimatization for 2 weeks, the experiment was started. The control group (C) were sacrificed 24 hours after being given a single dose of saline, intraperitoneally (ip) (150 mg/kg) on the seventh day of the experiment. The rats in the second group (CP) were sacrificed 24 hours after being given a single dose of Cyc, ip (150 mg/kg) on the seventh day of the experiment. The rats in third group (SP+CP) received *Spirulina* (1000 mg/kg bw/day) orally for 7 days and were sacrificed 24 hours after being given a single dose of Cyc (150 mg/kg ip) on the seventh day of the experiment. They were anesthetized with 50 mg/kg ketamine and 10 mg/kg xylasize before sacrifice by exsanguination. The kidneys and the urinary bladders were collected for histologic examination and immunohistochemical, biochemical, and terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL) assays.

Tissue Homogenization

Fresh tissues were washed with ice-cold phosphate-buffered saline (PBS) solution (10 mM Na_2HPO_4, 10 mM KH_2PO_4, 0.9 g NaCl/100 mL, pH 7.4) and weighed. After weights were recorded, homogenization was done with a tissue homogenizator (Heidolph DIA $\times$ 900, Schwabach, Germany) in ice-cold PBS immediately (1 mL/mg-volume/weight tissue) and they were kept at -70°C until assayed.

Measurement of MDA

Samples were thawed and centrifuged. Supernatants were used for the measurements. MDA assay was performed with a spectrophotometric assay (Catalog #NWK-MDA01, Northwest Life Science Canada, Vancouver, WA). The assay was based on the reaction of MDA with thiobarbituric acid (TBA), forming an MDA-TBA2 complex, which absorbs light strongly at 532 nm. The absorbance was directly proportional to the concentration MDA present. Intra-assay coefficient of variability (CV) was 3.2% and interassay CV was 2.5%. Data were expressed in nmol of MDA per 1 g of tissue.

Measurement of SOD ACTIVITY

Homogenates were thawed and centrifuged. Superoxide dismutase enzyme activity was measured immediately. Enzyme activity was measured by a colorimetric assay of superoxide dismutase (Catalog #NWK-SOD2, Northwest Life Science-Canada). The assay was based on monitoring the autoxidation rate of hematoxylin. In the presence of SOD enzyme, the rate of autoxidation is inhibited and the percentage of inhibition is linearly proportional to the amount of SOD present within a specific range. Sample SOD activity is determined by measuring the ratios of autoxidation rates in the presence and absence of the sample. The assay had an intra-assay CV of 8% and interassay CV of 12%. Data were expressed in U SOD per 1 g of tissue.

Measurement of Catalase Activity

The measurement of the catalase activity in the tissue homogenates were performed with a colorimetric assay. In this assay, the decomposition of peroxide is monitored at 240 nm (Catalog #NWK-SOD2, Northwest Life Science-Canada). The absorbance of hydrogen peroxide at 240 nm is measured directly to calculate the reaction rate because water and oxygen do not absorb at this wavelength. In the presence of catalase, the reaction rate is proportionally enhanced. The assay has an