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Modulatory effect of curcumin against genotoxicity and oxidative stress induced by cisplatin and methotrexate in male mice

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ABSTRACT

The use of dietary antioxidants to modulate the toxic side effects induced by the anticancer drugs used in chemotherapy is currently eliciting considerable interest. This study was undertaken to investigate the possible protective role of the antioxidant curcumin (CMN) against genotoxicity, cytotoxicity and oxidative stress induced by cisplatin and methotrexate. Male mice were administered CMN orally in the dosages of 60, 90, and 120 mg/kg for three consecutive days before a single intraperitoneal injection of either cisplatin (6.5 mg/kg) or methotrexate (10 mg/kg). Animals were sacrificed 24 h after treatment with the used anticancer drugs. The protective role of CMN against the genotoxic and cytotoxic effects of the tested anticancer drugs was evaluated by using micronucleus and comet assay. The oxidative stress induced by the two anticancer drugs was assessed by determining malondialdehyde and reduced glutathione levels in kidney tissues. The results indicated that CMN pretreatment at the tested doses reduced the incidence of micronuclei and DNA damage induced by cisplatin and methotrexate. Moreover, malondialdhyde level was significantly decreased while glutathione level was significantly increased in CMN pretreated groups compared with cisplatin- and methotrexate-treated groups. This protective effect of CMN could be attributed to its ability to scavenge reactive oxygen species.

1. Introduction

During the past decades, anticancer drugs have begun to offer major hope for chemical control of cancer. Unfortunately, several reports indicated the genotoxic and carcinogenic potential of the anticancer drugs that may lead to the formation of secondary cancer. The majority of anticancer drugs is not target specific and cannot distinguish between tumor cells and normal non-cancerous cells, particularly the proliferative ones. For this reason, even the most effective anticancer drugs may cause unwanted side effects. Extensive research has been done in order to develop effective and less toxic anticancer drugs or to use natural antioxidants that may reduce the unwanted side effects of existing drugs (Choudhury et al., 2000a).

Cisplatin and methotrexate (MTX) are two widely used drugs in cancer chemotherapy. They are effective in the treatment of several types of human cancer including testicular, ovarian, lung cancers and various types of sarcomas and carcinomas (Keshava et al., 1998; Bodo et al., 2005; Brabec and Kasparova, 2005; Padmanabhan et al., 2009). However, the use of these drugs in clinical practice is limited due to their undesirable side effects, such as nephrotoxicity. It has been shown in various studies that administration of both drugs causes serious alterations in the kidney functions characterized by signs of injury such as changes in urine volume, in body weight, in glutathione status, increase of lipid peroxidation products and changes in creatinine clearance. Moreover these drugs caused myelosuppression, gastrointestinal toxicity, ototoxicity, hepatotoxicity and neurotoxicity (Aftab et al., 1992; Choudhury et al., 2000b; Weijl et al., 2004). Moreover, these drugs are also highly genotoxic, inducing cytogenetic damage as well as cytostatic activity in many test systems (Osanto et al., 1991; Krishnaswamy and Dewey, 1993; Antunes et al., 2000). There is evidence that oxidative stress and the formation of reactive oxygen species is responsible for the toxic effects of these drugs (Jordan and Carmo-Fonseca, 2000; Widemann and Adamson, 2006).

Dietary antioxidants may enhance the anticancer effects of chemotherapy by decreasing or preventing certain side effects resulted from anticancer drugs (Mora et al., 2002). Recently, many

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authors focused their attention on the study of anticarcinogenic and antimutagenic effects of polyphenols to ameliorate the toxic side effects of anticancer drugs (Arjumand and Sultana, 2011; Serpeloni et al., 2013; Palipoch et al., 2014). Among these polyphenols, curcumin, a yellow pigment obtained from rhizomes of *Curcuma longa,* a major component of turmeric is a commonly used spice, which has been shown to possess many medicinal properties including immunomodulatory, antioxidant, antimutagenic and anticarcinogenic effects (Kawamori et al., 1999; Murray and Pizzorno, 1999). It has been reported to scavenge reactive oxygen species, inhibit lipid peroxidation and protect cellular macromolecules, including DNA, from oxidative damage (Fiorillo et al., 2008). Phase I clinical trials have shown that curcumin was safe even at high doses (12 g/day) (Anand et al., 2007).

Moreover, *in vivo* studies have demonstrated that curcumin inhibited the genotoxicity and oxidative stress of several anticancer drugs (Premkumar et al., 2004; Tirkey et al., 2005; Huang et al., 2011).

Hence, the present study has been carried out to investigate the possible protective effects of pretreatment with curcumin on the genotoxicity, cytotoxicity and oxidative stress induced by cisplatin and MTX in mammalian system. Different doses of curcumin were used to determine the most effective dose that exerts maximum protection. Two parameters were used to assess the protective role of curcumin against the genotoxic potential of cisplatin and MTX. Micronucleus assay was employed to measure chromosome damage in mice bone marrow cells. Comet assay (single-cell gel electrophoresis) was used as a parameter for measuring DNA damage in both kidney and bone marrow cells of mice. The percentage of polychromatic erythrocytes (PCEs) was also investigated to assess the effect of curcumin pretreatment on the cytotoxic effects of cisplatin and MTX. Some biochemical parameters were investigated to assess the protective role of curcumin against oxidative stress induced by the two tested anticancer drugs. These parameters include determination of malondialdhyde (MDA) and reduced glutathione (GSH) level in kidney cells.

2. Materials and methods

2.1. Experimental animals

Male CD-1 mice aged 6–8 weeks old (weighting 25 ± 2 g) were used. They were purchased from the Shistosome Biological Supply Program (SBSP), Theodor Bilharz Research Institute, Giza, Egypt. Animals were kept in plastic cages for 7 days before being treated to be accommodated with our laboratory conditions in a room maintained at temperature 23 ± 2 °C with a 12 h light/dark cycle. Food and water were supplied *ad libitum.*

2.2. Chemical agents

Cisplatin (CIS) was purchased from local pharmacies under the trade name cisplatin Mylan (Oncotec pharma produktion GmbH-Germany) and administered intraperitoneally at dose level of 6.5 mg/kg body weight. Methotrexate (MTX) was purchased from local pharmacies under the trade name methotrexate Mylan manufactured (Haupt Pharma GmbH 69800-France). It was injected as a single intraperitoneal dose (10 mg/kg b.w.). Curcumin (CMN) was purchased from Sigma Chemical Co. (St Louis, MO, USA, CAS No. 458-37-7). It was prepared suspended in saline just before the experiments and administered orally by gavage for three consecutive days at three dose levels, namely 60, 90 and 120 mg/kg body weight.

2.3. Treatment schedule

In all the experiments, fifty mice were randomly assigned to ten groups (five animals per group) for both CIS and MTX. Group 1 was injected *i.p.* with distilled water (0.1 ml/10 g b.w.) and used as negative control. Group 2 were administered CMN orally by gavage (120 mg/kg) for three consecutive days and sacrificed 24 h after the last treatment. Group 3 was treated *i.p.* with 6.5 mg/kg b.w. CIS (CIS group). Groups 4–6 were treated orally with CMN (60, 90 and 120 mg/kg), respectively for three consecutive days before CIS administration. Group 7 was injected *i.p.* with MTX (10 mg/kg b.w.). Groups 8–10 were treated orally with CMN (60, 90 and 120 mg/kg), respectively for three consecutive days before MTX injection. Animals were sacrificed 24 h after anticancer drugs injection. The above-mentioned doses of compound were selected based on preliminary studies.

2.4. Cell sample preparation

At the end of the experiment, the animals of each group were killed by cervical dislocation and their kidneys were carefully dissected and stored at −70 °C for the performance of comet assay and the biochemical determination of tissue malondialdehyde (MDA) and glutathione (GSH) levels. Bone marrow from femur bones was collected for micronucleus and comet assays.

2.5. Micronucleus assay (MNs)

Animals were killed by cervical dislocation and bone marrow smears were prepared and stained with May- Grünwald-Giemsa according to Schmid (1975). 2000 polychromatic erythrocytes (PCEs) were scored per animal and the number of micronucleated polychromatic erythrocytes (MNPCEs) was recorded in a blinded fashion. Slides were scored blindly using a light microscope with a 100× immersion objective. In addition, the number of PCEs among 2000 total erythrocytes per animal was recorded to evaluate the cytotoxicity of bone marrow and data were expressed as mean % PCEs ± SD.

2.6. Comet assay

Determination of DNA damage by comet assay was conducted according to Singh et al. (1988). A freshly prepared suspension of isolated bone marrow cells and kidney cells was mixed (1:1) with 85 μL of a low melting point agarose (0.5%, Sigma-Aldrich Co) dissolved in phosphate buffer saline (PBS) and spread onto microscope slides pre-coated with a normal melting point agarose (1%). The cells were covered with a cover slip and maintained at a temperature of 4 °C for 5 min. After the agarose gel had solidified, slides were immersed for 2 h at 4 °C in the dark in a lysing solution, consisting of 100 mM EDTA, 2.5 M NaCl, 10 mM Tris-HCl adjusted to pH 10 with 1% Triton X-100 and 10% DMSO, added just prior to use. Prior to electrophoresis, slides were removed from the lysing solution and placed for 20 min in a horizontal electrophoresis unit (near the anode) filled with freshly prepared alkaline buffer in order to allow the unwinding of DNA and to express alkali-labile damage. The electrophoresis alkaline solution consisted of 1 mM EDTA and 300 mM NaOH, pH > 13. After the unwinding of DNA electrophoresis was carried out in the same solution for 20 min at 25 V (300 mA). After electrophoresis, the alkali in the gels was neutralized by the rinsing of slides with Tris buffer (0.4 M Tris-HCl, pH 7.5). The DNA was then exposed for 5 min to absolute ethanol in order to preserve all the Comet assay slides. Subsequently, the slides were air-dried and stored at room temperature until scored for DNA migration. Each slide was stained with 50 μL ethidium