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ORIGINAL ARTICLE

Molecular and biochemical investigations on the effect of quercetin on oxidative stress induced by cisplatin in rat kidney

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Abstract The present study was aimed to investigate the ability of quercetin (QE) to ameliorate adverse effects of cisplatin (Cis.) on the renal tissue antioxidants by investigating the kidney antioxidant gene expression and the antioxidant enzymes activity. Forty rats divided into. Control rats. QE treated rats were orally administered 100 mg QE/kg for successive 30 days. Cis. injected rats were administered i.p. Cis. (12 mg/kg b.w.) for 5 mutual days. Cis. + QE rats were administered Cis. i.p. (12 mg/kg) and orally administered 100 mg QE/kg for consecutive 30 days. The obtained results indicated that Cis. induced oxidative stress in the renal tissue. That was through induction of free radical production, inhibition of the activity of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR) as well their genes expression. At the same time, vitamin E, vitamin C and reduced glutathione (GSH) levels were decreased. QE had the ability to overcome cisplatin-induced oxidative stress through the reduction of free radical levels. The antioxidant genes expression and antioxidant enzymes activity were induced. Finally the vitamin E, vitamin C and GSH levels were increased. Our work, proved the renoprotective effects of QE against oxidative stress induced by cisplatin.

1. Introduction

Cisplatin is the best and potent chemotherapeutic agent. Cis. is the front-line therapy for treatment of many tumors such as, ovarian, testicular, cervical, lunge and penile cancer. Cis. therapeutic effects are dose dependant. However, high dose of cisplatin therapy is limited due to its neuro-toxic and nephro-toxic effects (Noori and Mahboobc, 2010). Neurotoxicity arises in 50% of patients treated with Cis. (Gulec et al., 2013). Reactive oxygen species (ROS) are continuously synthesized in mitochondria. At the same time, mitochondria have potent ROS scavenge enzymes such as SOD, CAT, GPx, GR and GST. It is known that, Cis. accumulates in kidney epithelial cells mitochondria (Santos et al., 2008). That induces the ROS synthesis and decreases the antioxidant enzymes activities

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and GSH depletion (Huang et al., 2001). The antioxidants have a positive action on the oxidative stresses in cisplatin-induced nephrotoxicity (Tsuji et al., 2009).

Quercetin, QE (3,3′,4′,5,7-pentahydroxyflavone) is a major class of polyphenolic flavonoid compounds; it represents 60–75% of flavonoid intake. QE possesses a strong antioxidant ability through scavenging of free radicals and binding transition metal ions, inhibiting LPO. QE protected the renal tissues against gentamicin-induced nephrotoxicity. QE ameliorated the histopathological alterations and normalized the kidney biochemical markers (Abdel-Raheem et al., 2009). It has been reported that, QE protects the renal tissues from the age-related NF-κB activity that induces the oxidative stress. In addition, QE protects the kidneys from the adverse effects of ischemia through induction of xanthine dehydrogenase enzyme and inhepation of xanthine oxidase (Faddah et al., 2012). QE significantly decreases LPO and improves the activity of CAT and SOD and also prevents glutathione depletion. QE protected the heart, kidney and liver, from the oxidative stress caused by deoxycorticosterone acetate salt. It normalized the plasma LPO, liver and heart GSH, GST and GPx activities, and improves kidney GST activity (Galisteo et al., 2004).

2. Materials and methods

2.1. Animals

Forty male albino rats, weighing 110 ± 20 g each, were housed in standard cages in groups of five animals per cage under controlled conditions (temperature 25 ± 0.5 °C, a 12:12 light/dark cycle), with food and water free access. All procedures of our experiment were approved by the Medical Research Ethics Committee of King Abdulaziz, University, Saudi Arabia. Forty rats divided into. Control rats. QE treated rats were orally administered 100 mg QE/kg for successive 30 days. Cis. injected rats were administered i.p. Cis. (12 mg/kg b.w.) for 5 mutual days. Cis. + QE rats were administered Cis. (12 mg/kg) and orally administered 100 mg QE/kg for consecutive 30 days.

2.2. Sampling protocol

At the end of experimental period, blood samples were collected from eye vein. They were used to obtain serum for measuring the kidney function parameters. Rats from all groups were killed by decapitation and kidneys were dissected rapidly, 100 mg samples were preserved in liquid nitrogen to be used for investigation of the expressions of SOD, CAT, GR, and GPx genes. Kidney tissue samples of 0.5 g each were homogenized in 5 ml of cold HEPES buffer, pH 7.2 and kept at −80 °C till further biochemical investigations.

2.3. Biochemical investigation

The creatinine and urea levels in serum were investigated with a specific kit (Spinreact, Bas GIRONA, Spain, cat. No. 1001111 and 1001332). Malondialdehyde (MDA) was analyzed by measuring the production of TBARS according to the method of Buege and Aust (1978) using TBARS assay kit (Cat. No. 10009055, Cayman, USA). Protein carbonyls were determined according to Loro et al. (2012). GSH and tGSH were determined in the kidney homogenate, using a kit supplied by Cayman (Cat. No. 703002, Cayman, USA) according to the manufacturer's instructions (Ellman, 1959). Total antioxidant capacity (TAC) was determined using a kit supplied by Bio-diagnostic (Cat. NO. TA 25 12, Giza, Egypt). Following the method of Koracevic et al. (2001), SOD activity was determined using Cayman SOD diagnostic kit (Cat. No. 706002, Cayman, USA). CAT activity was determined using a kit (Cat. No. NWK-CAT01) purchased from Northwest Life Science Specialties (NWLSS), Vancouver, Canada, following the manufacturer's instructions (Aebi, 1984). GR activity was investigated following the method of Beutler (1969) using a kit supplied by NWLSS (Cat. No. NWK-GR01). GPx was determined using a kit (Cat. No. NWK-GPX01) purchased from NWLSS following the manufacturer's instructions (Lawrence and Burk, 1979).

2.4. Molecular analysis

Kidney SOD, CAT, GR and GPx genes expression were quantified using real time PCR. Total RNA was isolated from tissue samples using the RNeasy Mini Kit Qiagen (Cat. No. 74104). 0.5 μg of total RNA, was used for production of cDNA using Qiagen Long Range 2 Step RT-PCR Kit, (Cat. No. 205920). Five μL of total cDNA was mixed with 12.5 μL of 2× SYBR® Green PCR mix with ROX from Bio-Rad and 10 pmol/μL of each forward and reverse primer for the measured genes. The house keeping gene β-actin was used as a constitutive control for normalization. Primer 3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) was used for primers designed, as per the published rats SOD, CAT, GR, GPx and β-actin genes sequences of NCBI database all primers were provided by Sigma Aldrich (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) (Table 1). AbiPrism 7300 (Applied Biosystems, USA) was used for carrying out the PCR reactions. The RNA concentration in each sample was determined from the threshold cycle (Ct) values. The mRNA expression levels were calculated relative to ß-actin gene mRNA levels using the $2^{-DD\ CT}$ method.

3. Results

The serum creatinine and urea levels were higher in the Cis. treated rats than the control rats, the Cis. + QE, and the QE administered rats. The MDA, and protein carbonyl levels in the kidney homogenate were higher in the Cis. injected rats than the control rats, the Cis. + QE, and the QE administered rats (Table 2). The GSH, vitamin C, vitamin E, TAC, and tGSH levels in the kidney tissue were lower in the Cis. injected rats than the control rats, the Cis. plus QE, and the QE administered rats (Table 3). The SOD, CAT, GR, and GPx enzymes activities and gene expressions in the kidney tissue were lower in the Cis. injected rats than the control rats, the Cis. plus QE, and the QE administered rats (Tables 4 and 5). Simultaneous administration of Cis. + QE significantly reduced the elevated serum creatinine, urea, MDA, and protein carbonyl in kidney tissue. In addition, they significantly increased the GSH, vitamin C, vitamin E, TAC, and tGSH levels in the kidney tissue. Moreover, they significantly induced the gene expression and activities of CAT, SOD, GR, and GPx enzymes in the kidney