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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

Table 1 Primer oligonucleotide sequences of GPx, CAT, CuZnSOD and ß-actin and GR genes.

Gene		Oligonucleotide sequences	Size (bp)	Gen ID
GPx	F	5′-CACAGTCCACCGTGTATGCC-3′	292	S50336.1
	R	5′-AAGTTGGGCTCGAACCCACC-3′		
CAT	F	5′-GTCCGATTCTCCACAGTCGC-3′	272	AH004967.1
	R	5′-CGCTGAACAAGAAAGTAACCTG-3′		
SOD	F	5′-ATGGGGACAATACACAAGGC-3′	225	Z21917.1
	R	5′-TCATCTTGTTTCTCGTGGAC-3′		
ß-Actin	F	5′-TCACTATCGGCAATGTGCGG-3′	260	NM_007393
	R	5′-GCTCAGGAGGAGCAATGATG-3′		
GR	F	5′-CCATGTGGTTACTGCACTTCC-3′	171	NM_053906
	R	5′-GTTCCTTTCCTTCTCCTGAGC-3′		

Table 2 Effect of cisplatin and/or Quercetin on serum creatinine, urea and kidney homogenate free radicals of Albino rat.

Parameter	Control	QE	Cisplatin	Cisplatin + QE
Serum creatinine (mg/dl)	0.92 ± 0.06	0.8 ± 0.08	2.9 ± 0.2** #	0.85 ± 0.01
Serum urea (mg/dl)	50 ± 4.2	48 ± 3.4	74.5 ± 10.4**,##	54 ± 1.8
Protein carbonyls (μmol g^{-1}wt.w)	1.8 ± 0.2	1.76 ± 0.25	8 ± 0.4** ##	1.85 ± 0.22
MDA (nmol g^{-1}wt.w)	0.85 ± 0.14	0.75 ± 0.2	3 ± 0.3** #	1 ± 0.3

MDA, malondialdehyde.
** $p < 0.01$, statistically significant difference from control group.
# $p < 0.05$, statistically significant difference from cisplatin + QE group.

Table 3 Effect of cisplatin and/or Quercetin on antioxidant substances in kidney homogenate of albino rat.

Parameter	Control	QE	Cisplatin	Cisplatin + QE
Vitamin E (mg/L homogenate)	624.9 ± 73	681.5 ± 243	214.7 ± 50*** #	565 ± 78.9
Vitamin C (mg/ml homogenate	25 ± 5.3	27.6 ± 5.2	12.8 ± 5.5** ##	24.9 ± 6
GSH (μmol g^{-1} wt.w)	165 ± 10	170 ± 13	76 ± 6** ##	155 ± 26
TAC (μM g^{-1} wt. w)	5.5 ± 0.5	6.03 ± 0.6	3 ± 0.4** ##	5.2 ± 0.2
Total glutathione (μmol g^{-1} wt. w)	173 ± 13	180 ± 15	101 ± 8.1** ##	165 ± 16
GSSG (μmol g^{-1} wt.w)	8 ± 0.9	10 ± 1.3	25 ± 2.5** ##	10 ± 1.4

GSH, reduced glutathione; TAC, total antioxidant capacity; wt.w; wet weight tissue.
** $p < 0.01$, statistically significant difference from control group.
*** $p < 0.001$, statistically significant difference from control group.
# $p < 0.05$, statistically significant difference from cisplatin + QE group.
$p < 0.01$, statistically significant difference from cisplatin + QE group.

Table 4 Effect of cisplatin and/or Quercetin on antioxidant enzyme activities in kidney homogenate of albino rat.

Parameter	Control	QE	Cisplatin	Cisplatin + QE
SOD (μmol/mg.wt.w/min)	20.3 ± 1.8	23 ± 2	8.2 ± 2** #	18 ± 1.2
CAT (μmol/H_2O_2 decomposed/mg.wt.w/min)	798 ± 15.2	801 ± 4.5	480 ± 10** ##	760 ± 25
GR (U/mg.wt.w)	5.3 ± 0.3	5.6 ± 0.6	2.8 ± 0.3**.#	5 ± 0.5
GPx (μmol/mg.wt.w/min)	130 ± 10	135 ± 5	46 ± 6** #	120 ± 10

SOD, superoxide dismutase; CAT, catalase enzyme; GR, glutathione reductase; GPx, glutathione peroxidase; wt.w, wet weight tissue.
*** $p < 0.001$, statistically significant difference from control group.
** $p < 0.01$, statistically significant difference from control group.
# $p < 0.05$, statistically significant difference from cisplatin + QE group.
$p < 0.01$, statistically significant difference from cisplatin + QE group.

Table 5 Effect of cisplatin and/or Quercetin on antioxidant gene expression in kidney tissue of albino rat.

Parameter	Control	QE	Cisplatin	Cisplatin + QE
SOD	1.1 ± 0.001	1.3 ± 0.011	0.55 ± 0.002$^{*,\#}$	0.95 ± 0.3
CAT	0.95 ± 0.002	1 ± 0.012	0.34 ± 0.001$^{**,\#\#}$	0.8 ± 0.2
GR	1 ± 0.003	1.13 ± 0.006	0.43 ± 0.03$^{*,\#}$	0.9 ± 0.05
GPx	1 ± 0.001	1.21 ± 0.01 5	0.6 ± 0.025$^{*,\#}$	1.2 ± 0.1

SOD, superoxide dismutase; CAT, catalase enzyme; GR, glutathione reductase; GPx, glutathione peroxidase; wt.w, wet weight tissue.
** $p < 0.01$, statistically significant difference from control group.
$^{\#}$ $p < 0.05$, statistically significant difference from cisplatin + QE group.
$^{\#\#}$ $p < 0.01$, statistically significant difference from cisplatin + QE group.

tissue. Administration of QE alone had no effect on any measured parameter.

4. Discussion

Cisplatin is the most used and effective tumor chemotherapeutic drug. The clinical use of cisplatin is limited by the onset of severe nephrotoxicity. The acute nephrotoxicity has occurred in 20–30% of patients treated with cisplatin (Ronald et al., 2010). In the recent years lots of researches have been made to overcome this problem especially regarding the optimum duration and dose. Various antioxidant compounds have been used to protect the kidneys from cisplatin nephrotoxicity, especially in experimental animal models (Penelope et al., 2011). Moreover, it is well documented that Cisplatin-induced nephrotoxicity is related to the reactive oxygen species (ROS). Therefore, the potential of antioxidant in nephrotoxicity induced by cisplatin has been tested. Many studies (Noori and Mahboobc, 2010) have mentioned that cisplatin elevated the levels of the kidney function biochemical markers such as serum urea and creatinine. Consistent with these data we observed in our study that serum creatinine and urea concentration were elevated denoting the damage of the renal glomeruli While, the concentrations of serum creatinine and urea were reduced in rats that were administered a combination of cisplatin + QE. These findings may be in concord with many other studies, which found that, antioxidant compounds like QE overcame the elevation in plasma creatinine and urea levels caused by cisplatin (Penelope et al., 2011). The elevated urea and creatinine concentrations in cisplatin injected rats could be attributed to the elevated ROS (Somani et al., 2000). The elevated ROS attacks the membrane lipids generating the lipid peroxides, which are manifested by increased MDA. The increased MDA in the renal tissue depleted vitamin E, vitamin C, and GSH. GSH is important in maintenance of the cell redox and cell membrane integrity. GSH plays an essential role in free radical scavenging through providing the proton for the antioxidant enzymes (Abdel-Raheem et al., 2009). In our work, GSH, and tGSH levels were decreased in the kidney homogenate after cisplatin administration. This finding is proved by other researchers, who have mentioned the reduction of the renal GSH level in response to ROS induced by cisplatin injection (Silva et al., 2001). The most convincing explanation to GSH reduction after cisplatin administration is the over GSH consumption in non-enzymatic removal of ROS. Additionally, the enzymatic oxidation of GSH with over production of GSSG by the oxidant radicals, with increasing of GSSG levels as seen in the present study. Pretreatment with antioxidant significantly increases the GSH and normalizes the GSSG levels in renal tissues. The recycling of GSSG to GSH is controlled by the enzyme GR. Therefore, cisplatin may be interfered with the GSH production by reduction of the enzyme GR activity and/or gene expression as proved in our results and confirmed by the result of Abdel-Raheem et al. (2009). It was reported that, QE potentiated the activities and gene expression of GR under stress condition (Ali et al., 2014), that enhances the recycling of GSSG back to GSH. Our findings were coordinated with this result as co-administration of cisplatin + QE induced the levels of GSH, GR activity and gene expression in comparison with cisplatin-treated rats. Furthermore, ciplatin induces rapid alterations in the composition of membranous lipids that could be initiated by free radicals. This is enforced by elevated MDA levels, the main lipid peroxidation end products, in ciplatin treated rat kidney (Lalila et al., 2001). We have observed an increase in MDA and protein carbonyl levels in the cisplatin administered rats, consistent with the previously mentioned studies. Otherwise, the expression of SOD, GR, CAT and GPx genes was reduced in rats that were treated with cisplatin alone as well as the SOD, GR, CAT and GPx enzymes activity was reduced. SOD is the enzyme that catalyzes superoxide radicals reduction to H_2O_2. This reaction has a 10,000-fold faster rate than spontaneous dismutation (Abdel-Raheem et al., 2009). Inhibition of CAT, SOD, and GPx enzymes activities and their gene expression in rats injected with cisplatin has been previously observed (Xin et al., 2007; Noori and Mahboobc, 2010). All these alterations were reversed in rats that were administered QE + cisplatin. QE obviously, induced CAT, SOD and GPx gene expressions as well as enhanced their enzyme activities. Additionally, QE reduced the increased MDA and protein carbonyl levels. This proves that QE administration overcomes the oxidative stress by its antioxidant properties.

5. Conclusion

This work revealed on a molecular level that, QE minimized the renal toxicity induced by cisplatin. QE decreased the serum creatinine and urea levels. QE improved nonenzymatic antioxidant substances as GSH, vitamin E and vitamin C. Additionally, it induced the enzymatic antioxidant system on the gene and protein levels. The protective effect of QE against renal damage induced by cisplatin could be explained by its antioxidant properties.

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