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Comparative study of multiple dosage of quercetin against cisplatin-induced nephrotoxicity and oxidative stress in rat kidneys

Estela B. Behling[1], Milena C. Sendão[1], Heloísa D.C. Francescato[2], Lusânia M.G. Antunes[2], Roberto S. Costa[3], Maria de Lourdes P. Bianchi[2]

[1]Departamento de Alimentos e Nutrição, Faculdade de Ciencias Farmaceuticas de Araraquara, UNESP, Rod. Araraquara/Jaú, km 01, 14801-902, Araraquara, SP, Brazil

[2]Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciencias Farmaceuticas de Ribeirão Preto, Universidade de São Paulo, Av. do Café, s/n, 14040-903, Ribeirão Preto, SP, Brazil

[3]Departamento de Patologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes, 3900, 14040-903, Ribeirão Preto, SP, Brazil

Correspondence: Lusânia M. G. Antunes, e-mail: lusania@fcfrp.usp.br

Abstract:
Quercetin, a typical bioflavonoid ubiquitously present in fruits and vegetables, is considered to be helpful for human health. Cisplatin (cDDP) is one of the most active cytotoxic agents in the treatment of a wide range of solid tumors. The aim of this study was to investigate the possible effect of quercetin, a bioflavonoid with antioxidant potential, on cisplatin-induced nephrotoxicity and lipid peroxidation in rats. Gavage administrations of water, propylene glycol and quercetin (50 mg/kg) were made 24 and 1 h before saline or cDDP (5 mg/kg) *ip* injections and were repeated daily for 2, 5 or 20 subsequent days. Rats were killed 2, 5 and 20 days after *ip* injections, and blood and urine samples were collected to determine plasma creatinine, urine volume and osmolality. The kidneys were removed to determine the levels of thiobarbituric acid-reactive substances (TBARS) and for histological studies. Cisplatin increased lipid peroxidation, urine volume and plasma creatinine levels and decreased urine osmolality. Treatment with quercetin attenuated these alterations. These results demonstrate the role of oxidative stress and suggest a protective effect of quercetin on cisplatin-induced nephrotoxicity in adult Wistar rats.

Key words:
quercetin, cisplatin, antioxidants, lipid peroxidation, nephrotoxicity

Abbreviations: ATN – acute tubular necrosis, cDDP – *cis*-diammine dichloroplatinum II, MDA – malondialdehyde, P – propylene glycol, Q – quercetin, TBARS – thiobarbituric acid-reactive substances.

Introduction

A large number of natural products and dietary components have been evaluated as potential chemoprotective agents. Flavonoids are phytophenolic compounds with strong antioxidant properties. Quercetin (3,5,7,3',4'-pentahydroxyflavone) is one of the most abundant flavonoids in the human diet, commonly present in most edible fruits and vegetables [16]. This bioflavonoid is a potent oxygen free radical scavenger and a metal chelator, capable of inhibiting lipid peroxidation in *in vitro* and *in vivo* systems [11, 12]. Even though pharmacokinetic and bioavailability information on quercetin is scarce and contradictory, it is reported to have many beneficial effects on human health including cardioprotection and anticancer, anti-

oxidant, antiulcer, antiallergic, antiinflammatory, antiviral and antiproliferative activities [27].

Cisplatin (cDDP; *cis*-diamminedichloroplatinum II) is a potent anticancer agent, especially used in the treatment of solid tumors. However, clinical use of the compound is often limited by its adverse effects, including renal impairment, intestinal toxicity and myelosuppression, of which renal toxicity is the most serious dose-limiting factor [23]. Signs of injury, such as changes in urine volume, osmolality, and reduction of the glomerular filtration rate characterize the alterations induced by cDDP in kidney function [22]. In kidney tissue, cDDP-induced nephrotoxicity is closely associated with increased lipid peroxidation [6]. Therefore, oxygen free radicals may play a central role in cDDP-induced renal injury.

Much attention has been given to the possible role of dietary antioxidants in protecting the kidneys against cDDP-induced nephrotoxicity. Many antioxidant compounds have been studied as chemoprotective agents such as vitamin C, curcumin, selenium, bixin, and other dietary components that scavenge free radicals formed by exposure to cDDP [2, 3, 31]. Quercetin has been shown to enhance the antiproliferative effects of cDDP *in vitro* [17, 29] and *in vivo* [18]. Therefore, combining cDDP with quercetin might be of therapeutic value.

Thus, the aim of this study was to investigate the protective effect of multiple doses of the bioflavonoid quercetin on cDDP-induced nephrotocixity and lipid peroxidation in adult Wistar rats.

Materials and Methods

Chemical agents

Cisplatin (cDDP; cis-diamminedichloroplatinum II; CAS no. 15663-27-1; Platinil) was purchased from Quiral Química do Brasil S/A, quercetin (CAS no. 6151-25-3) from Sigma Chemical Co. (St. Louis, MO, USA) and propylene glycol from Galena Química e Farmaçęutica Ltda (Brazil). All other chemicals and reagents used were of the highest commercial grade available.

Animals and experimental protocols

Male Wistar rats (*Rattus norvegicus*) weighing 200–220 g were housed in hanging stainless steel cages under controlled temperature and lighting (24°C; 12 h light-12 h dark cycles, respectively). Food and fresh distilled water were provided *ad libitum*. This study has been approved by the Animal Ethics Committee from the University of São Paulo, Campus of Ribeirão Preto. The rats were acclimatized for 2 days and divided into six different experimental groups consisting of six animals each, set up as follows: negative control (C, 1.0 ml of distilled water given by gavage before 1.0 of saline *ip*), propylene glycol (P, 1.0 ml given by gavage before 1.0 ml of saline *ip*), quercetin (Q, 50 mg/kg, dissolved in a propylene glycol vehicle given by gavage before 1.0 ml of saline *ip*), cisplatin (cDDP, 1.0 ml distilled water given by gavage before cDDP 5 mg/kg *ip*), P + cDDP (1.0 ml of propylene glycol given by gavage before cDDP *ip*) and Q + cDDP (quercetin in a propylene glycol vehicle given by gavage before cDDP *ip*).

Gavage administrations were made 24 and 1 h prior to the cDDP *ip* injection and repeated for 2, 5 or 20 consecutive days. The optimal dosages and timing for the bioflavonoid treatments (50 mg/kg) were determined in previous studies [13]. Two, five or twenty days after the injection, the rats were killed by decapitation and the kidneys were immediately removed for morphological studies and determinations of thiobarbituric acid-reactive substances (TBARS). Blood samples were collected to determine plasma creatinine. Urine volume and osmolality were determined in the last 24 h collection.

Plasma creatinine levels, urine volume and osmolality

Blood samples collected from sectioned neck vessels were used to determine plasma creatinine levels using a commercial kit (Labtest, Lagoa Santa, MG, Brazil). Urine volume was measured by the last 24 h collection before the rats were killed and its osmolality was determined by freezing point depression (Fiske OS Osmometer, Needham Heights, Mass., USA).

Morphological studies

The abdominal cavity of the sacrificed rats was opened, the left renal vascular pedicle was dissected

and the left kidney was immediately removed and transversally sectioned. A 1 cm^2, 5 mm thick slice was fixed in methacarn for 4 h, rinsed with alcohol 70° and processed for paraffin embedding. Histological sections 3 μm thick were stained with hematoxylin and eosin, and examined under a light microscope.

The glomerular, tubular, interstitial and vascular compartments of the renal parenchyma were examined to detect changes. Necrosis in the lining epithelium, presence of intratubular casts, dilatation of the tubular lumen and presence of mitoses were determined in tubules from the cortex, outer and inner medulla. The results were scored according to the extent of damage: 0 = no damage; 1 = up to 25% of the tubular area showing acute tubular necrosis (ATN); 2 = 25–50% of the tubular area showing ATN; 3 = 50–75% of tubular area showing ATN; 4 = more than 75% of the tubular area showing ATN. Acute tubular necrosis was scored semiquantitatively on the basis of the sum of casts, necrosis and tubular dilatation.

Lipid peroxidation in rat kidney

The malondialdehyde (MDA) content, a measure of lipid peroxidation, was assayed as thiobarbituric acid-reactive substances (TBARS) in renal tissue from rats sacrificed 2 days after cDDP *ip* injection [32]. Slices were homogenized in ice-cold 1.15% KCl, and 0.5 ml aliquots of the homogenate were mixed with 3 ml of 1% phosphoric acid and 1 ml of 0.6% thiobarbituric acid. The mixture was heated for 45 min in a boiling water bath after adding 4 ml of *n*-butanol was vigorously vortexed and centrifuged at 1200 × g for 20 min. The absorbance of the upper organic layer at 535 and 520 nm was measured with a diode array spectrophotometer (Spectronic Instruments Genesys II) and compared with a standard of freshly prepared 1,1,3,3-tetraethoxypropane, at the concentrations of 5.125, 10.25 and 20.5 nmol/ml. TBARS were expressed as nanomoles of malondialdehyde (MDA) per milligram of renal tissue.

Statistical analysis

Data concerning urinary volume and osmolality (V, Uosm), plasma creatinine (Pcreat) and TBARS were expressed as the means ± SEM and analyzed statistically using the Kruskal-Wallis nonparametric test followed by the Dunn post-test. Analysis of variance with multiple comparisons were made to evaluate significance of morphological data and using the Newman Keuls test. The level of significance was $p < 0.05$.

Results

The effects of multiple doses of quercetin on cDDP-induced nephrotoxicity were evaluated by measuring the changes in urine volume and osmolality and in plasma creatinine levels. The cDDP-treated group produced a significantly higher urinary volume and its osmolality was decreased 5 and 20 days after cDDP administration as compared with the control group (Tab. 1) ($p < 0.05$). These changes were inhibited when cDDP-treated rats received multiple doses of quercetin ($p < 0.05$) (Tab. 1).

The effects of cDDP treatment on plasma creatinine levels are also shown in Table 1. There was not

Tab. 1. Effect of quercetin on renal function in animals killed 5 and 20 days after cDDP *ip* injection

	C	P	Q	cDDP	P + cDDP	Q + cDDP
5 days						
V	7 ± 0.57	5.83 ± 1.77[b,c]	8.33 ± 1.17[c]	30.33 ± 0.33[a]	30.50 ± 1.66[a]	19 ± 1.21
U_{osm}	1768 ± 43.97	1763 ± 237.4[b,c]	1708 ± 68.77[b]	587.3 ± 12.12[a]	676 ± 19.35[a]	797.5 ± 23.34
P_{creat}	0.66 ± 0.02	0.67 ± 0.08[b]	0.70 ± 0.06[b]	1.57 ± 0.19[a]	0.95 ± 0.09	0.68 ± 0.03[b]
20 days						
V	11.33 ± 1.98	12.67 ± 1.36[b]	16.33 ± 2.85	29.83 ± 1.33[a]	26.0 ± 3.04[a]	20.17 ± 2.16
U_{osm}	1735 ± 134.1	1543 ± 43.5[b,c]	1620 ± 110.0[b,c]	723.3 ± 43.37[a]	786.8 ± 53.1[a]	997.7 ± 49.58

Each value represents the mean ± SEM of six animals. C – control group; P – propylene glycol group; Q – quercetin group; cDDP – cisplatin group; P+cDDP – propylene glycol + cisplatin group; Q + cDDP – quercetin + cisplatin group. V – Urinary volume (ml/24 h); U_{osm} = urine osmolality (mOsm/kg H_2O); P_{creat} – plasma creatinine (mg/dl). [a] Significant differences from control group ($p < 0.05$); [b] significant differences from cDDP group ($p < 0.05$). [c] significant differences from P + cDDP group ($p < 0.05$) Kruskal-Wallis and Dunn tests

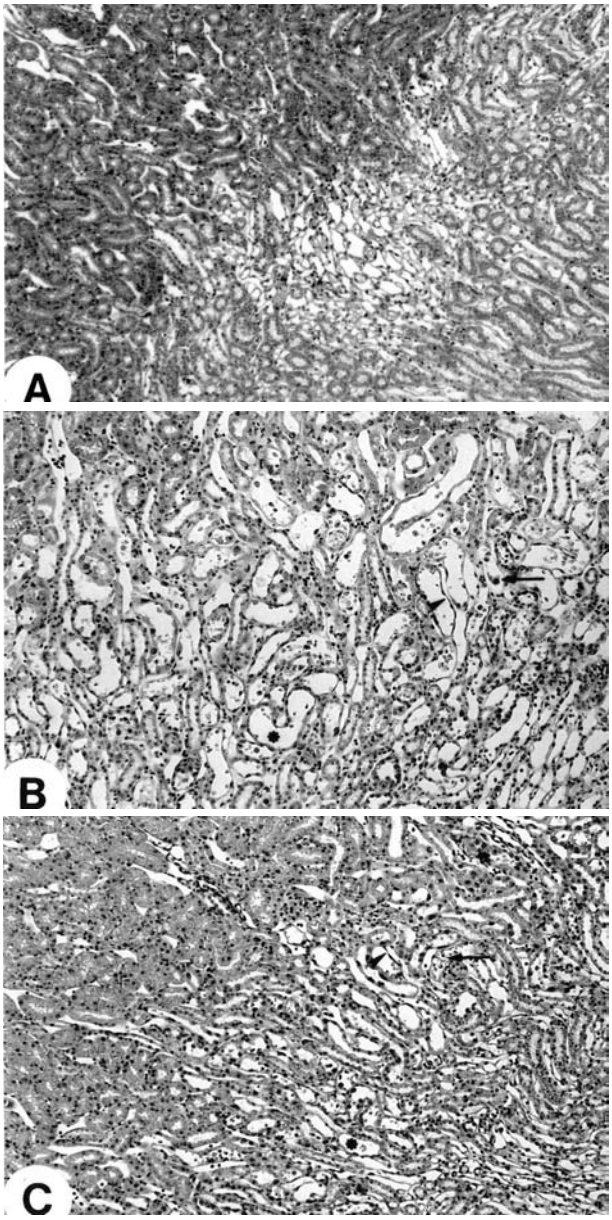

Fig. 1. Photomicrographs of hematoxylin and eosin stained kidney sections from rats from the control group showing normal tubules (**A**). (**B**) Cisplatin (cDDP)-treated rat kidney showing acute tubular necrosis characterized by intense tubular dilatation (*), descamation of cells into tubular lumen (arrow), and denudation of the tubular basement membrane (arrow head). (**C**) Quercetin + cDDP-treated rat kidney showing attenuation of the histological changes induced by cDDP on day 5 after the treatment. Original magnification A × 200, B and C × 400

a significant difference in plasma creatinine levels in the Q and Q + cDDP groups when compared with the control ($p > 0.05$). However, animals in the cDDP-treated group had elevated plasma creatinine values as compared with the control, Q and Q + cDDP groups ($p < 0.05$). Quercetin treatment was able to restore plasma creatinine to control levels in cDDP-treated animals ($p < 0.05$).

Tab. 2. Renal malondialdehyde levels in animals killed 2 days after cDDP *ip* injection

Groups	MDA (nmol/g tissue)	% of control
C	131.1 ± 6.39	100.0
P	122.7 ± 4.2	93.6
Q	115.9 ± 6.53[b]	88.4
cDDP	179.7 ± 8.8[a]	137.1
P + cDDP	154.1 ± 9.45	117.5
Q + cDDP	116.2 ± 7.28[b]	88.6

Each value represents the mean ± SEM of six animals. C – control group; P. – propylene glycol group; Q – quercetin group; cDDP – cisplatin group; P + cDDP – propylene glycol + cisplatin group; Q + cDDP – quercetin + cisplatin group. [a] Significant differences from control group, $p < 0.05$, [b] significant differences from cDDP group, $p < 0.05$. Kruskal-Wallis and Dunn tests

Histological changes in the kidney were evaluated. Histological picture of the kidney tissue the histological was normal, with score of grade 0 seen in the C, P and Q groups (Fig. 1A). Morphological light microscopy studies of the renal cortex and outer medulla of rats killed on days 5 and 20 after cDDP injection showed ATN features such as: tubular cell necrosis, focal areas of denuded basement membrane, intraluminal casts, swelling and flattening of proximal tubular cells with brush border loss, diffuse interstitial edema, and interstitial inflammatory cell infiltrates. Animals killed 5 days after the cDDP injection presented intense and diffuse lesions (score 4), which can be seen in Figure 1B. These alterations were significantly attenuated ($p < 0.05$) by treatment with Q + cDDP (Fig. 1C, score 2 ± 0.73) when compared with cDDP and P + cDPP groups. Animals killed on day 20 after cDDP injection, again showed histological alterations characteristic of chronic nephropathy such as interstitial fibrosis, tubular atrophy and dilatation and inflammatory cell infiltrates. These changes were less intense in the animals treated with Q + cDDP and killed on day 20, when compared with the cDDP group ($p < 0.05$). Quercetin or vehicle administered alone did not affect any of the structural parameters investigated (Tab. 3).

The effect of quercetin on lipid peroxidation caused by cisplatin in rat kidney tissue was examined by determining TBARS. The administration of a single dose of cDDP (5 mg/kg) increased the formation of lipid peroxides when compared with the control group. However, lipid peroxidation was significantly

Tab. 3. Effect of quercetin treatment on morphological changes as assessed by histopathological examination of kidneys from rats killed 20 days after *ip* cDDP injection

	C	P	Q	cDDP	P + cDDP	Q + cDDP
1	0	0	0	3	2	1
2	0	0	0	4	3	1
3	0	0	0	4	4	2
4	0	0	0	4	4	3
5	0	0	0	4	4	3
6	0	0	0	4	4	4
Mean ± SEM	0	$0^{b,c}$	$0^{b,c}$	$3.83^{a} \pm 0.16$	$3.5^{a} \pm 0.34$	2.33 ± 0.49

C – control group; P– propylene glycol group; Q – quercetin group; cDDP – cisplatin group; P + cDDP – propylene glycol + cisplatin group; Q + cDDP – quercetin + cisplatin group. Tubular degenerations were scored as follows: 0 – no damage; 1 = up to 25% of the tubular area showing acute tubular necrosis (ATN); 2 = 25–50% of the tubular area showing ATN; 3 = 50–75% of tubular area showing ATN; 4 = more than 75% of the tubular area showing ATN. [a] Significant differences from control group, ($p < 0.05$), [b] significant differences from cDDP group ($p < 0.05$), [c] significant differences from P + cDDP group ($p < 0.05$). Kruskal-Wallis and Newman Keuls tests. Data are expressed as the mean ± SEM of six animals in each group

reduced in the animals treated with Q + cDDP when compared with the cDDP-treated group ($p < 0.05$) (Tab. 2). The TBARS content was similar in the control, Q and Q + cDDP groups ($p > 0.05$).

Discussion

Development of therapies to prevent the action or the generation of oxygen free radicals may influence the progression of renal oxidative stress, along with the appearance of cDDP-induced acute renal failure. Protective effects against cDDP-induced nephrotoxicity have been reported for extracts of natural products and dietary antioxidants [1, 7]. In this study, we carried out a comparative investigation of multiple doses of quercetin on the cDDP-induced nephrotoxicity and oxidative stress in rat kidneys.

The alterations in renal function observed in rat models correlate well with the nephrotoxic effects of cDDP in patients treated with this antitumor agent [8]. In the present investigation, a single dose of cDDP, (5 mg/kg, *ip*), in rats resulted in the deterioration of renal function, tubular necrosis and increased renal lipid peroxidation. Quercetin administered in daily oral doses of 50 mg/kg before and after cDDP treatment either prevented or significantly reduced the development of cDDP-induced acute renal failure as evidenced by functional and morphological findings.

A decreased glomerular filtration rate, evidenced by increased plasma creatinine levels, has been observed following cDDP administration [4]. It has been previously shown that cDDP predictably lowers glomerular filtration rates in a dose-dependent manner even after a single-dose exposure [33]. Literature reports have shown that this increase is transitory, returning to normal levels a few days after the cDDP injection [13]. Data presented here also show that plasma creatinine levels increased 5 days after cDDP administration when compared with the control groups. Multiple doses of quercetin associated with cDDP prevented this change in renal function in rats.

The kidneys accumulate and retain platinum complexes to a greater extent than other organs, perhaps via mediated transport, and it is the main excretory outlet for either intravenous or intraperitoneal cDDP [4, 21]. In this study, cDDP-induced renal impairment included an increase in urinary volume and a decrease in osmolality. This is in agreement with Antunes et al. [3]. The polyuria observed 5–7 days following cDDP administration may be due to impairments in the proximal tubular and thick ascending limb of Henle without changing the response of the papillary collecting duct to antidiuretic hormone [30]. Quercetin treatment along with cDDP administration in rats could attenuate these alterations.

cDDP acts mostly on the proximal renal tubule of the kidney. Proximal tubular epithelial cells take up this antitumor agent and this actively leads to higher concentrations than those found in the plasma. cDDP toxicity in proximal tubular cells is morphologically characterized by tubular necrosis [4]. The morphological data show that by day 5, cDDP caused structural alterations in the renal cortex and outer medulla, characteristic of acute tubular necrosis, and by day 20, histological features of chronic nephropathy such as interstitial fibrosis, tubular atrophy and dilatation. While renal injury was apparent in both groups 5 days after cDDP injection, the proximal tubular injury was less intense in the group treated with Q + cDDP. This protection was also observed when other bioflavonoids were used in this model of renal injury. Dobyan et al. [10] observed the protective effects of O-(β-hydroxyethyl)-rutoside in rats treated with cDDP. Ozbek et al. [26] reported that caffeic acid phenethyl ester prevented cDDP-related nephrotoxicity,

at least in part, because of its antioxidant properties. The persistence of acute lesions up to 20 days after cDDP administration was reported by Gonzalez-Vitale et al. [15]. This could be explained by the fact that renal excretion of cDDP is extremely slow [9], thus exposing the kidney tubular epithelium to a continuous prolonged deleterious effect of this antitumor agent.

The underlying mechanism of cDDP-induced nephrotoxicity is still not well known but many recent *in vitro* and *in vivo* studies indicate an important role for the reactive oxygen metabolites in the pathogenesis of this effect [22]. It has been suggested that lipid peroxidation is the major mechanism linked to cDDP-induced nephrotoxicity. However, other mechanisms may be involved in the deleterious effect of cDDP on kidney cells. In the present study, the administration of cDDP resulted in renal lipid peroxidation, determined by a TBARS increase when compared with the control and quercetin-treated groups. Quercetin significantly attenuated the increase in TBARS concentration in renal tissue, probably because of its capacity to scavenge oxygen free radicals in the kidney tubular cells of rats.

The mechanisms by which quercetin protects against the cDDP´s cytotoxicity has not been completely elucidated. The pronounced effect of quercetin on lipid peroxidation supports previous findings suggesting that quercetin directly interferes with nonenzymatic lipid peroxidation [20]. There have been numerous other reports on the structure-activity relationships of flavonoid antioxidants [25]. Among the several factors affecting the antioxidative activity of flavonoids, hydroxyl groups on the B-ring seem to play the most important role [27, 25]. It is reported that among structurally homologous flavonoids, oxygen scavenging capacity increases as the total number of hydroxyl groups increases [5].

Concurrent administration of quercetin and cyclosporine reduced the elevated levels of TBARS and attenuated renal dysfunction and morphological changes in rats [28]. Even though quercetin is conjugated during the absorption process, the conjugates still seem to retain antioxidant activity [19]. In experimental renovascular hypertension, a daily oral dose of quercetin has also been shown to normalize plasma nitrates, nitrites and TBARS concentrations, in addition to exerting an antihypertensive effect [14]. It is possible that quercetin protects the kidneys by many other mechanisms, since it has been recently demonstrated that this bioflavonoid prevents chronic cadmium-induced nephrotoxicity by overexpressing endothelial nitric oxide synthase and cyclooxygenase-2 in rats [24].

In conclusion, we have shown that experimental cDDP administration in rats is associated with an increased lipid peroxidation, urine volume and plasma creatinine and a decreased urine osmolality. The present results suggest that quercetin is a potentially effective chemoprotective agent by acting in the kidneys as a potent scavenger of free radicals, thus preventing the toxic effects of cDDP both at the biochemical and histological levels.

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