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Differential effect of quercetin on cisplatin-induced toxicity in kidney and tumor tissues

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ABSTRACT

Strategies to minimize the nephrotoxicity of platinated antineoplastics without affecting its antitumour efficacy are strongly necessary to improve the pharmacotoxicological profile of these drugs. The natural flavonoid quercetin has been shown to afford nephroprotection without affecting cisplatin antitumour effect. The purpose of the present study has been to assess the differential mechanisms of action of cisplatin and quercetin on kidney and tumour tissues that could explain these effects. Wistar rats bearing subcutaneous tumours were treated with cisplatin and quercetin (and the appropriate controls). Tumour size and renal function evolution was monitored during 6 days. Platinum and quercetin content were also determined in both tissues. All the parameters studied, including blood supply, inflammation, apoptosis, critical MAPK signaling and oxidative stress in the cisplatin-treated animals are almost normalized by quercetin in the kidneys, but unaffected in the tumours. Our results suggest that in a cancer model *in vivo*, the protection exerted by quercetin on cisplatin nephrotoxicity is related to its antioxidant, vascular, anti-inflammatory and antiapoptotic effects, but these properties do not affect the mechanisms responsible for the antitumour effect of cisplatin.

1. Introduction

The platinated antineoplastic cisplatin is known to cause acute kidney injury (AKI) in 25–35% of the therapeutic courses (Sato et al., 2016). The kidneys accumulate cisplatin in the renal tissue more than in any other organs, resulting in necrosis of the proximal renal tubules and apoptosis in the distal nephron (Kawai et al., 2005; Ma et al., 2016).

In most cases, cisplatin-induced AKI is mostly mild and reversible, although a number of patients need to be transitorily dialyzed

Abbreviations: ang II, angiotensin II; ANOVA, analysis of variance; AKI, acute kidney injury; C, Control group; CHAPS, 3-((3-cholamidopropyl)dimethylammonio)-1-propanesulfonate; CP, Cisplatin group; CP + Q, Cisplatin-Quercetin group; Cr_{pl}, plasma creatinine; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; EGTA, egtazic acid; ELISA, Enzyme-Linked ImmunoSorbent Assay; EMSA, electrophoretic mobility shift assay; EPR, enhanced permeability and retention; ERK, extracellular signal–regulated kinase; $FENa^+$, sodium fractional excretion; GSH, reduced glutathione; GSSG, oxidized glutathione; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HPLC-DAD/MS, high performance liquid chromatography coupled to a diode array detector and mass spectrometer; ICP-MS, Inductively Coupled Plasma Mass Spectrometry method; JNK, c-Jun N-terminal kinase; Kim-1, kidney injury molecule-1; MDA, malondialdehyde; MPO, myeloperoxidase activity; NAG, N-acetyl-β-D-glucosaminidase; NF-κB, nuclear Factor κB; iNOS, inducible nitric oxide synthase; p38MAPK, p38 mitogen-activated protein kinase; PAF, platelet activating factor; Q, Quercetin group; ROS, reactive oxygen specie; SEM, standard error of mean; TNF-α, tumour necrosis Factor α.

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(Menczer et al., 1991), or go through chronic renal fibrosis and progressive renal dysfunction, especially at high dosage or upon repeated administration (Kawai et al., 2009). Even transitory AKI is not a trivial event, it is associated to an increased short, medium and long term morbimortality (Chertow et al., 2005; Odutayo et al., 2017). Accordingly, for human and economic reasons, better prevention of drug nephrotoxicity poses an urgent need.

The severity and clinical importance of chemotherapy-associated nephrotoxicity have led to increasing research on new strategies to reduce this side effect, including vigorous hydration with saline during cisplatin treatment in patients (Launay-Vacher et al., 2008). Many molecules proved successful at preventing or softening nephrotoxic AKI, including that induced by cisplatin, in preclinical studies conducted in animal models or cell lines (Ali and Al Moundhri, 2006; Chirino and Pedraza-Chaverri, 2009; Sanchez-Gonzalez et al., 2011a; Mundhe et al., 2015). However, either they have not progressed to the clinical setting or they have failed to demonstrate a clear protective effect after meta-analysis evaluation. This is the case of antioxidants (Dashti-Khavidaki et al., 2012), despite oxidative stress being a mechanism of cytotoxicity activated by many drugs, including cisplatin (Rashid et al., 2013. In other cases, renoprotection was also associated to inhibition of the drug's therapeutic effect, which voids its clinical application.

In this line, interest in dietary flavonoids has greatly increased recently, owing to their antioxidant and anti-inflammatory properties. Quercetin is the most abundant flavonol in the Mediterranean human diet (Heim et al., 2002). It is a potent reactive oxygen species (ROS) scavenger with many reported health benefits, including cardioprotection and anticancer, antiproliferative, antioxidant and anti-inflammatory properties (Vicente-Sánchez et al., 2008; Bischoff, 2008; Boots et al., 2008). Quercetin has been shown to ameliorate cisplatin nephrotoxicity *in vitro* (Kuhlmann et al., 1998) and *in vivo* (Behling et al., 2006; Francescato et al., 2004). Moreover, quercetin synergistically enhances the antiproliferative and apoptotic effects of cisplatin on cancer cells (Borska et al., 2004; Jakubowicz-Gil et al., 2005; Sharma et al., 2005; Zhang et al., 2008). *In vivo*, interestingly, a previous study from our laboratory demonstrated that quercetin protects from cisplatin-induced renal damage without compromising the effect of cisplatin on reducing tumour size (Sanchez-Gonzalez et al., 2011b). On these grounds, the purpose of the present study was to assess the differential mechanisms of action of cisplatin and quercetin on kidney and tumour tissues in order to explain why quercetin protects renal tissue from the toxic effects of cisplatin with no effect on the antitumoural properties of the drug.

2. Materials and methods

Except where otherwise indicated all reagents were purchased from Sigma-Aldrich (Madrid, Spain). Cisplatin (1 mg/mL) was dissolved in 0.9% saline, and quercetin (50 mg/mL) in saline with 0.16% Tween-20.

2.1. Animals and experimental design

Male Fischer F344 rats (weighing approximately 200 g) were used (Charles River Laboratories, Barcelona, Spain). All procedures were approved by the Bioethics Committee for Animal Care and Use of the *Universidad de Salamanca* (Law 32/2007/Spain and RD 266/1998/CyL) and complied with the Guide for Care and Use of Laboratory Animals (Directive, 2003/65/CE). Animals were housed 6 *per* cage, and in individual metabolic cages after cisplatin inception. Pilot assays and our previous studies (Morales et al., 2006a; Sanchez-Gonzalez et al., 2011b) were used to select the dosage of cisplatin and quercetin. All rats were injected in the dorsal area with 2×10^6 tumour cells (Rat 13762 Mat B-III breast adenocarcinoma, LGC Promochem, Barcelona, Spain). Seven days later, once tumours were visible and had grown to 100–200 mm^3, animals were randomly divided into four groups: *Control group (C)*, animals that received physiological saline containing 0.16% Tween-20 (the vehicle for quercetin) daily for 9 days (100 μL/100 g, i.p.), starting from day 7 after tumour implant. *Quercetin group (Q)* animals received quercetin (50 mg kg^{-1} once a day for 9 days i.p.), beginning from day 7 after inoculation of tumour cells. *Cisplatin group (CP)* animals received a single dose of cisplatin (4 mg kg^{-1} i.p.), starting from day 10 after tumour implant. *Cisplatin-Quercetin group (CP + Q)* animals received cisplatin on day 10 after tumour implant, and quercetin 4 days before and 5 days after cisplatin administration (Fig. 1). Tumour growth was monitored with a digital caliper. Tumour volumes were calculated according to the formula: $V = a \times b^2 \times 0.52$, where a is the largest superficial diameter and b is the smallest superficial diameter. For ethical reasons, animal with tumours reaching 3 cm in diameter underwent mandatory sacrifice. Plasma (from tail blood) and 24-h urine samples were collected in metabolic cages every other day beginning after cisplatin treatment and kept at −80 °C until use. At the end of the experiment (day 2 or day 6 after cisplatin administration), animals were exanguinated under anaesthesia and the kidneys and tumours were rapidly dissected, immediately frozen and kept at −80 °C until use.

2.2. Renal function studies

Plasma creatinine (Cr_{pl}) and urea concentrations and urinary sodium and *N*-acetyl-β-D-glucosaminidase (NAG) were measured as previously described (Morales et al., 2006b). Sodium fractional excretion ($FENa^+$) was calculated using standard formulae (Morales et al., 2002). Kidney injury molecule (Kim-1) was detected by Western blot. Western blot was performed with 100–150 μg tissue extracts or 21 μL urine as previously described (Ichimura et al., 2004; Morales et al., 2006a). Membranes were probed with *anti*-KIM-1/TIM-1 (R&D Systems, Minneapolis, MN, USA). The membranes from tissue samples were also reprobed with mouse monoclonal *anti*-β-actin (Sigma-Aldrich, St. Louis, MO, USA) antibody to verify equal loading of protein in each lane.

2.3. Oxidative stress

Lipid peroxide content was assayed in the form of thiobarbituric acid-reactive substances (TBARS) by the colorimetric method of Recknagel, 1983 using Malondialdehyde (MDA) as standard. H_2O_2 and peroxidase activity in postmitochondrial supernatants were measured using a commercial *Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit* (Molecular Probes, Eugene, OR, USA), according to the manufacturer's instructions. Reduced and oxidized glutathione (GSH and GSSG) was assayed by the fluorometric method described by Hissin and Hilf (1976). For preparation of postmitochondrial supernatants, kidney and tumour tissues were homogenized in *buffer A* [250 mM Sucrose, 1 mM egtazic acid (EGTA), 20 mM Trizma-HCl, pH 7.2] containing 2 mg/mL of lipid-free albumin. The homogenates were cleared by centrifugation and further was centrifuged at 11,000 g for 10 min (4 °C) to get the postmitochondrial supernatant.