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Tangeretin attenuates cisplatin-induced renal injury in rats: Impact on the inflammatory cascade and oxidative perturbations

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ABSTRACT

Background: Despite the efficacy of cisplatin as a chemotherapeutic agent against various cancers, its clinical utility is limited by serious adverse reactions including nephrotoxicity.
Aim: The current study aims to investigate the protective potential of tangeretin, a citrus flavone with marked antioxidant actions, against cisplatin-induced renal injury in rats.
Methods: Tangeretin was administered at 50 and 100 mg/kg p.o. for 1 week starting one day before cisplatin (7.5 mg/kg i.p.) injection. Likewise, silymarin was administered at 100 mg/kg orally. Renal function tests, histopathology, oxidative stress and inflammatory events were investigated.
Results: Tangeretin mitigated the increased levels of serum creatinine, blood urea nitrogen and histopathologic alterations evoked by cisplatin. It alleviated renal oxidative stress due to cisplatin by lowering lipid peroxides, nitric oxide and Nrf2 levels with concomitant enhancement of GSH and GPx. Tangeretin also suppressed the upregulated inflammatory response seen with cisplatin treatment by down-regulation of activated NF-κB p65 protein expression together with its downstream effectors e.g., iNOS and TNF-α, with restoration of the anti-inflammatory interleukin IL-10. Additionally, it down-regulated the expression of caspase-3, an apoptotic marker, thus favoring renal cell survival. Importantly, tangeretin enhanced the cytotoxic actions of cisplatin in Hep3B and HCT-116 human cancer cell lines.
Conclusion: Together, these findings accentuate the dual benefit of tangeretin: mitigation of renal injury-induced by cisplatin and enhancement of its cytotoxic effects.

1. Introduction

Cisplatin (*cis*-diammine-dichloro-platinum) has been frequently considered as a first choice chemotherapeutic agent for the treatment of a wide variety of solid tumors, including ovarian, testicular, bladder, head and neck, esophageal, and small cell lung cancers [1]. However, cisplatin affords dose and time-dependent renal toxicity which is manifested by increased serum creatinine and blood urea nitrogen (BUN), thus limiting its clinical use in 25–35% of patients undergoing therapy [2]. Therefore, the search for new adjunct therapies that can mitigate cisplatin toxicity during the cancer battle is critically warranted [1].

Mounting evidence indicated that the molecular mechanisms underlying cisplatin-induced renal injury are multifactorial. Among them, accumulation of cisplatin in renal parenchyma, along with the interference with nuclear and mitochondrial DNA and mitochondrial respiratory chain are regarded as major players [3]. Meanwhile, the generation of reactive oxygen species (ROS) and the decline of renal antioxidant defenses, which, in turn trigger apoptosis in renal tubular cells have been closely linked to the deleterious effects of cisplatin [4]. The pathogenesis of cisplatin-

Abbreviations: BUN, blood urea nitrogen; GSH, glutathione; GPx, glutathione peroxidase; H&E, hematoxylin and eosin; IL-10, interleukin-10; iNOS, inducible nitric oxide synthase; MDA, malondialdehyde; MPO, myeloperoxidase; NF-κB, nuclear factor kappa B; Nrf2, nuclear factor erythroid 2-related factor; NO, nitric oxide; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α.

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evoked nephrotoxicity also involves the surge of proinflammatory cytokines e.g., tumor necrosis factor-α (TNF-α) together with the activation of nuclear factor-κB (NF-κB) and inducible nitric oxide synthase (iNOS) pathways [5].

Recently, the use of natural anti-oxidants such as flavonoids has become a promising approach to protect against cisplatin-induced renal injury [2,6]. Tangeretin, a polymethoxy citrus flavone, has displayed remarkable antioxidant features via direct ROS scavenging and indirect boosting of cellular glutathione [7]. It has a great advantage over other chemically related flavones as it exhibits considerable intestinal absorption and thus has better bioavailability [8]. It is also safe following oral administration in laboratory animals [9]. Tangeretin has been reported to protect against DMBA-induced rat mammary carcinoma [7,10], streptozotocin-induced diabetic rats [11] and D-galactosamine-induced liver injury in rat [12]. Interestingly, tangeretin has demonstrated significant cytotoxic actions against cancer cell proliferation in a diverse range of human cancer cell lines including squamous cell carcinoma, gliosarcoma, leukemia and melanoma [13]. In the same context, our research group has previously reported that tangeretin sensitizes cisplatin-resistant ovarian cancer cells to low doses of cisplatin via downregulation of the PI3K/Akt survival pathway [13]. However, no previous study has addressed the impact of tangeretin on cisplatin-induced nephrotoxicity *in vivo*. Thus, the current study investigates the potential of tangeretin to protect against cisplatin-induced renal injury in rats.

2. Material and methods

2.1. In vivo assays

2.1.1. Animals

Adult male Wistar rats weighing 120–150 g were purchased from the National Research Centre (Cairo, Egypt). Animals were maintained at 25 ± 2 °C, under a 12/12 h light-dark cycle and allowed free access to water and food. The experimental protocol was approved by the Committee of Animal Care and Use of Faculty of Pharmacy, Beni-Suef University, according to the recommendations of the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1996).

2.1.2. Drugs and chemicals

Tangeretin was purchased from Shaanxi Huike Botanical Development Co. (China). Cisplatin, silymarin, thiobarbituric acid, Ellman's reagent, vanadium trichloride, *N*-(1-Naphtyl) ethylenediamine dihydrochloride and sulfanilamide were purchased from Sigma-Aldrich (USA). All chemicals used during the study were of the finest analytical grade.

2.1.3. Experimental design

Rats were randomly allocated into 7 groups, each of 8 rats, as follows:

(1) Control group: normal rats which received 2% Tween 80 orally for one week and a single i.p injection of isotonic saline on the second day.
(2) Control + TG 100 group: normal rats which received 100 mg/kg tangeretin in 2% Tween 80 orally for one week and a single i.p injection of isotonic saline on the second day.
(3) Control + SIL group: normal rats which received 100 mg/kg silymarin in 2% Tween 80 orally for one week and a single i.p injection of isotonic saline on the second day.
(4) CIS group: 2% Tween 80 was administered orally for one week and a single i.p injection of 7.5 mg/kg of cisplatin on the second day [14].
(5) CIS + TG 50 group: received 50 mg/kg of tangeretin in 2% Tween 80 orally for one week [15,16] and a single i.p. dose of 7.5 mg/kg cisplatin on the second day 1 h after tangeretin administration.
(6) CIS + TG 100 group: received 100 mg/kg of tangeretin in 2% Tween 80 orally for one week [11] and a single i.p. dose of 7.5 mg/kg cisplatin on the second day 1 h after tangeretin administration.
(7) CIS + SIL group: received 100 mg/kg of silymarin in 2% Tween 80 orally for one week [17] and a single i.p. dose of 7.5 mg/kg cisplatin on the second day 1 h after silymarin administration.

2.1.4. Preparation of samples

At the end of the experiment, rats were fasted overnight, blood samples were collected and serum was separated by centrifugation for 10 min at 3000 r.p.m. to be used for the assessment of kidney function tests. Then, animals were sacrificed and kidneys were immediately dissected out and washed with cold saline. One kidney was homogenized in 10 vol of ice-cold lysis buffer, centrifuged at 1000×*g* for 15 min and the supernatants were used for subsequent measurement of TNF-α, IL-10, GSH, lipid peroxides, nitric oxide, Nrf2 and GPx levels. The other kidney was immersed in 10% formol saline for the histopathology and immunohistochemical detection of NF-κB, iNOS and caspase-3.

2.1.5. Histopathologic examination

Kidney samples were fixed in 10% formol saline for 24 h, and paraffin blocks were processed as described earlier [18]. Sections were stained with hematoxylin-eosin (H&E) and visualized using a Nikon microscope at a magnification of 400×. Sections were examined by a skilled observer who was experimentally blinded.

2.1.6. Determination of kidney function tests

Serum creatinine concentration and blood urea nitrogen (BUN) were determined according to the manufacturer procedures of the assay kits purchased from Diamond Diagnostics (Egypt).

2.1.7. Investigation of kidney oxidative stress markers

Lipid peroxides were measured as previously described [19] and expressed as malondialdehyde (MDA) level. Nitric oxide (NO) was measured as total nitrate/nitrite (NOx) content and was detected using a Nitric Oxide (NO_2^-/NO_3^-) assay Designs kit (MI, USA) according to manufacturer procedures. Reduced glutathione (GSH) was estimated as described earlier [20] whereas glutathione peroxidase (GPx) activity was determined using a Sigma-Aldrich assay kit (MD, USA) according to the manufacturer procedures. Nuclear factor erythroid 2-related factor (Nrf2) was determined using a Total Nrf2 Cell-Based Colorimetric ELISA Kit (DE, USA) according to manufacturer procedures.

2.1.8. Investigation of kidney inflammatory signals (TNF-α and IL-10)

The levels of TNF-α and interleukin 10 (IL-10) were determined by RayBio Rat ELISA kits (GA, USA) according to manufacturer instructions. Briefly, samples were added into a 96-well plate coated with antibodies specific for Rat TNF-α or IL-10, followed by an HRP-conjugated streptavidin solution. A substrate solution was finally added to the wells and the developed color was measured at 450 nm.

2.1.9. Immunohistochemical detection of inflammatory (NF-κB p65 and, iNOS) and apoptotic (caspase-3) proteins

Immunohistochemical detection of target proteins was