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Intermediate filament protein expression pattern and inflammatory response changes in kidneys of rats receiving doxorubicin chemotherapy and quercetin

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ABSTRACT

The aim of this study was to explore the potential effects of quercetin (QUR) on doxorubicin (DOX)-induced nephrotoxicity. Fifty male rats were assigned to five groups (10 rats each): a control group, a DOX-treated group (total dose, 15 mg/kg bw, intraperitoneally), a QUR-treated group (50 mg/kg bw/day, orally), a prophylaxis co-treated group, and a therapeutic co-treated group. Biochemical parameters and renal function were measured. Moreover, kidney tissues were homogenized for inflammatory marker evaluation and real-time qPCR analysis to determine the changes in intermediate filament protein mRNA levels (desmin, vimentin, connexin 43 and nestin). QUR exhibited a significant nephroprotective effect, particularly when it was administered prior to and simultaneously with DOX treatment (prophylaxis co-treated group). This role was biochemically demonstrated by the significant modulation of DOX-induced body weight loss, hypoproteinemia, and elevated serum creatinine and urea. Moreover, QUR attenuated the inflammatory response as shown by decreased renal nitric oxide, tumor necrosis factor-α production and myeloperoxidase activity elicited by DOX injection. These biochemical improvements were accompanied by a significant histopathological restoration of rat kidney tissue and successful down-regulation of the intermediate filament protein mRNA levels, indicating amelioration of DOX-induced podocyte injury. Taken together, these results conclusively demonstrated that QUR administration has a prophylactic effect on DOX-induced injury in the rat kidney.

1. Introduction

Doxorubicin (DOX) is an antitumor anthracycline antibiotic that is therapeutically used either alone or in combination with other anticancer drugs in the treatment of various neoplastic diseases, such as prostate cancer, hematological malignancies, bile duct neoplasms and cancers of the stomach, esophagus, uterus, and liver (Bonadonna et al., 1970; Carvalho et al., 2009). The anticancer effects of DOX in tumor cells primarily target the nucleus, mitochondria, and biological membranes and induce acute cellular changes, such as DNA structural alterations, inhibition of topoisomerase I and II, and free radical generation. Consequently, cell death and promotion of senescence-like growth arrest occur (Box, 2007).

Anticancer chemotherapy generally disrupts physiological homeostasis and influences various organs during treatment (Ayla et al., 2011). Hence, the successful clinical use of DOX has been limited because of its toxicities and its various noxious effects; the most evident is cardiotoxicity (Chatterjee et al., 2010), which has been extensively investigated in previous studies (Zakaria et al., 2018; Abbas and Kabil, 2017). In addition to cardiomyopathy, other effects, such as myelotoxicity and hepatotoxicity, may potentially contribute to DOX-induced nephrotoxicity. DOX-induced kidney damage characterized by glomerular and tubulointerstitial inflammation and renal fibrosis, similar to the effects observed in nephritic patients (Pereira Wde et al., 2014). Previous evidence has shown that the DOX-induced nephrotoxicity may be mediated by free radical generation and iron-dependent oxidative injury of biological macromolecules and mitochondria (Liu et al., 2007; Ayla et al., 2011; Giampieri et al., 2016), which consequently lead to inflammation and damage to podocytes and play a role in the pathogenesis of progressive renal damage (Yang et al., 2009; Guo et al., 2014; Xu et al., 2016).

The mechanisms of podocyte injury have attracted increasing interest, especially with regard to the external layer of the glomerular basement membrane, which is indispensable for maintaining the integrity of glomerular filtration barrier. Thus, protection against podocyte damage has been recognized as a possible therapeutic strategy to

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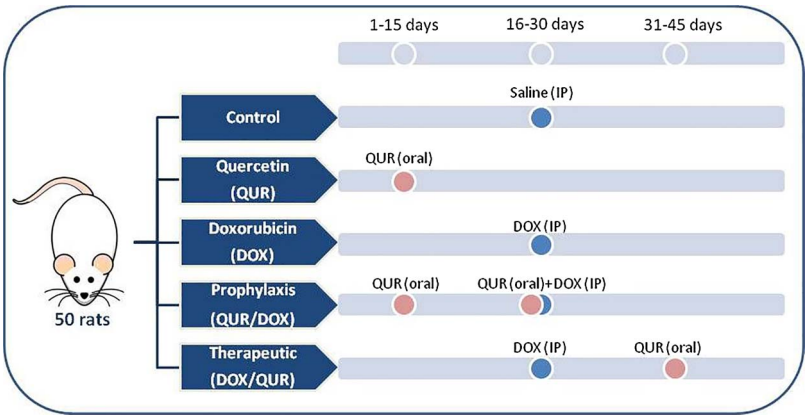

Fig. 1. Experimental groups and treatments: QUR (50 mg/kg bw/day for 15 days, orally) and DOX (15 mg/kg bw, in six injections over 2 weeks, intaperitoneally).

maintain normal renal function in the treatment of renal disorders (Shi et al., 2013).

There is a need for supportive therapy with improved efficacy in the treatment of renal inflammation and podocyte injury induced by DOX to minimize the danger of renal failure as well as to improve therapeutic regimens with DOX chemotherapy.

Quercetin (QUR) is a common flavonol found in various fruits and vegetables; the highest QUR content has been found in onions, asparagus, red leaf lettuce, apples, cherries and berries, with lower levels in broccoli, peas, green peppers, and tomatoes (USDA, 2003). QUR exerted efficient protective actions against various toxic insults and demonstrated neuroprotective efficacy (Lakroun et al., 2015; Gasmi et al., 2017; Joseph, 2015), cardioprotective effects (Zakaria et al., 2018), and protection of other tissues (Verma et al., 2017; Alam et al., 2017; Gerin et al., 2016). The underlying mechanisms involve antagonizing oxidative stress-mediated damage and free radical scavenging potency (Cho et al., 2006). Moreover, this molecule exhibits anti-inflammatory and cytoprotective efficiency (Hayashi et al., 2012; Gerin et al., 2016). QUR could suppress inflammatory responses by inhibiting the AMPK/TXNIP (Zhang et al., 2014), NF-κB (Kang et al., 2013) and MAPK/AP-1 (Endale et al., 2013) signaling pathways.

Furthermore, QUR has been investigated for its ability to protect against kidney damage induced by nephrotoxic insult and diseases (Faddah et al., 2012; Gomes et al., 2014; Chaudhary et al., 2015), which show improvements in morphological and functional outcomes with QUR. QUR protected renal tissue during ischemia and reperfusion by maintaining higher levels of the enzyme xanthine dehydrogenase relative to the enzyme xanthine oxidase, which was decreased (Sanhueza et al., 1992).

Given the results of previous reports, this study was conducted to investigate the effectiveness of QUR in either preventing or treating DOX-induced nephrotoxicity and to promote renal function in rats. This was accomplished by biochemical evaluation of kidney functions, inflammatory markers, and histopathological changes, and we also assessed the relationship between regulation of intermediate filament protein expression and modulation of the induced renal damage

2. Material and methods

2.1. Tested compounds and chemicals

QUR powder (purity ≥95%) was purchased from Sigma-Aldrich Chemical Company, Saint Louis, MO, USA. DOX hydrochloride powder was obtained from Pharmacia Italia (SPA, Italy). All other chemicals used in the investigation were of analytical grade and obtained from Sigma–Aldrich Chemical Company.

2.2. Animal grouping and treatment

Fifty male Sprague–Dawley rats (150–200 g), which were obtained from the laboratory animal farm at the Faculty of Veterinary Medicine, Zagazig University, were used in the experiment. The animals were kept under hygienic conditions and housed in cages with a photoperiod of 12 h light/12 h dark cycle and a relative humidity of 50% at 22–28 °C. Water and feed were accessible *ad libitum* throughout the acclimatization (2 weeks) and experimental periods. The experiment was conducted according to the general rules of the National Institutes of Health (NIH) for the Care and Use of Laboratory Animals in scientific investigations and affirmed by the Ethics of Animal Use in Research Committee (EAURC), Zagazig University, Egypt.

Animals were randomly assigned to five groups (n = 10). The control group was comprised of rats that were intraperitoneally (IP) injected with physiological saline (three times a week, for two weeks). The QUR-treated group was orally administered QUR at a dose of 50 mg/kg bw for 15 days via gastric tube (Sanchez-Gonzalez et al., 2011). The DOX-treated group was injected intraperitoneally (IP) with DOX at a total dose of 15 mg/kg bw divided into 6 injections, and each animal received 2.5 mg/kg bw/injection, three times a week, for two weeks (Siveski-Iliskovic et al., 1994). For the prophylaxis co-treated group (QUR/DOX), rats orally received QUR for 15 days prior to and simultaneously with DOX treatment (QUR was given 1 h before DOX injection). The therapeutic co-treated group (DOX/QUR) orally received QUR for 15 days after the treatment with DOX, at the same dose and route described above (Fig. 1). At the end of the experiment, final body weight was recorded for each animal in all groups for calculation of body weight changes.

2.3. Tissue collection and homogenate preparations

The blood samples were collected from the median canthus (orbital vessels) of control and treated rats without anticoagulant and then centrifuged at 3000 rpm for 15 min for separation of serum, which was stored at −20 °C for biochemical analysis of kidney function markers. Following animal sacrifice, kidney tissue specimens were dissected and rinsed with sterile physiological saline and assigned to three sets; one set was snap frozen by immersion in liquid nitrogen and kept at −80 °C until further processing for gene expression analysis. Another set was homogenized for 5 min in 0.115 M PBS (1:5 w/v) and centrifuged at 3000 rpm for 15 min at 4 °C, and the supernatant was aspirated and utilized for analysis of inflammatory markers. The final set of kidney tissues was fixed in 10% neutral buffered formalin for histopathological studies.