International Immunopharmacology 28 (2015) 500–506

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Renoprotective mechanisms of morin in cisplatin-induced kidney injury

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ARTICLE INFO

Article history:
Received 18 March 2015
Received in revised form 6 July 2015
Accepted 8 July 2015
Available online 25 July 2015

Keywords:
Cisplatin
Kidney injury
Morin
Oxidative stress
Inflammation
Apoptosis

ABSTRACT

In this study, we investigated the renoprotective effects of morin on cisplatin-induced kidney injury in mice. Serum creatinine and blood urea nitrogen (BUN) levels, glutathione peroxidase (GSH-PX) and superoxide dismutase (SOD) activities were determined according to the corresponding kits. The mRNA levels of TNF-α and IL-1β in kidney tissues were measured by quantitative real-time PCR (qRT-PCR). The activities of cytochrome P450 2E1 (CYP2E1), nuclear factor kappa B (NF-κB) p65, P38 mitogen-activated protein kinase (MAPK), Bax, p53 and cleaved caspase 3 were evaluated by western blotting. The results showed that the model of cisplatin-induced kidney injury was successfully replicated, and morin significantly attenuated histopathological changes and decreased the levels of TNF-α and IL-1β in the kidneys. In addition, morin attenuated the activation of CYP2E1, phospho-NF-κB p65, phospho-P38 MAPK, Bax, phospho-p53 and cleaved caspase 3 in CP-induced kidney injury. In conclusion, these results indicated that the renoprotective mechanisms of morin may be attributed to the suppression of oxidative stress, inflammation and apoptosis in CP-induced kidney injury.

1. Introduction

Cisplatin (cis-diamminedichloroplatinum(II), CP) is widely used in treating different kinds of cancers, including those of the head and neck, ovarian, cervical, and lung [1,2]. The anticancer mechanism of cisplatin is widely thought to have been interacted with DNA and the formation of cross-links, thereby arresting DNA synthesis and replication [3,4]. Although cisplatin has been a mainstay for cancer therapy, its clinical utility is limited by severe side effects in normal tissues, such as nephrotoxicity, neurotoxicity, ototoxicity and allergic reactions [2,3]. A variety of approaches have been attempted to curtail or eliminate side effects, but nephrotoxicity remains a major obstacle in cisplatin chemotherapy. Cisplatin nephrotoxicity is recognized as a complex process involving various factors and signaling pathways, and strategies to prevent cisplatin nephrotoxicity have primarily focused on reduced renal cisplatin accumulation or activation, anti-oxidants, anti-inflammation or anti-apoptosis. While there are some common approaches to prevent nephrotoxicity, combination cisplatin with chemical composition of herb medicine has drawn great attention these past few years.

Morin (3,5,7,2′,4′-pentahydroxyflavone; Fig. 1), a flavone originally isolated from fruits and Chinese herbs, possesses various biological properties such as anti-inflammation, anti-oxidation and anti-mutagenesis [5,6]. In addition, morin also exerted anti-cancer property through suppression of the signal transducer and activator of transcription 3 (STAT3) pathway [7]. Previous studies demonstrated that natural compounds, such as luteolin and chlorogenic acid showed renoprotective properties against CP-induced kidney injury [8,9]. However, the effects of morin on CP-induced kidney injury remain unclear. The aim of this study was to examine the effects of morin on CP-induced kidney injury and elucidate its molecular mechanisms.

2. Material and methods

2.1. Chemicals and antibodies

Cisplatin was purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Morin was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) and was free of endotoxin. GSH-PX Assay Kit and Superoxide Dismutase Assay Kit were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). phospho-P65 rabbit polyclonal antibody, P65 rabbit polyclonal antibody, phosphor-p38 MAPK rabbit polyclonal antibody, p38 MAPK rabbit polyclonal antibody, Bax rabbit antibody, phospho-p53 rabbit polyclonal antibody, cleaved caspase 3 rabbit antibody, β-actin antibody and secondary antibodies were acquired from Cell Signaling Technology Inc. (Beverly, MA, USA).

2.2. Animals

Male BALB/cN mice, 8 weeks old, weight 23–26 g, were purchased from the Center of Experimental Animals of Baiqiuen Medical College

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http://dx.doi.org/10.1016/j.intimp.2015.07.009
1567-5769/

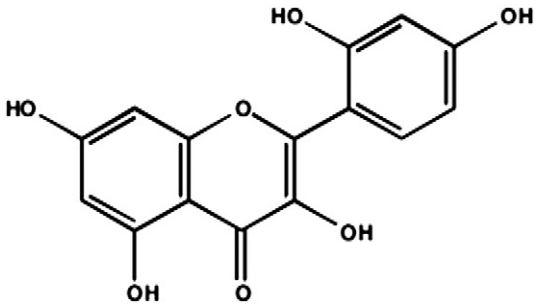

Fig. 1. Chemical structure of morin.

of Jilin University (Jilin, China). Mice were fed the respective diets and have free access to tap water. All experiments were approved by the Jilin University Animal Care and Use Committee.

2.3. Experimental design

Mice were randomly divided into 6 groups with 5 animals per group. Group I (control group) received saline and Group VI was treated with morin (40 mg/kg) dissolved in saline. Group II received CP (20 mg/kg) diluted with 5% DMSO (v/v) as a single intraperitoneal injection. Groups III, IV and V were intraperitoneally administered daily with morin at a dose of 10, 20 and 40 mg/kg respectively for 3 consecutive days, 72 h after intraperitoneal injection with cisplatin (20 mg/kg). Four days after cisplatin injection, mice were sacrificed by cervical dislocation. Blood was collected from retro-orbital sinus, and serum was separated to examine BUN and serum creatinine levels. One kidney was removed to determine histological analysis and the other one was stored at −80 °C for later biochemical analysis.

2.4. Kidney function analysis

The levels of creatinine and BUN (Changchun Huili Biotechnology Co., Ltd., Jilin, China) in the blood were determined by creatinine and BUN assay kits according to the manufacturer's protocols.

2.5. Histological analysis

The kidney tissues were collected and fixed in 10% formalin for histological analysis. Then, these kidney tissues were embedded in paraffin after dehydration, sectioned and stained with hematoxylin and eosin (H&E). Histopathological changes in the kidneys were assessed by scoring necrotic epithelial cells, cellular vacuolization and cast formation under a light microscope in 10 different fields.

2.6. GSH-PX and SOD analyses

GSH-PX level in kidney tissue was determined by GSH-PX Assay Kit. SOD activity was examined by Superoxide Dismutase Assay Kit according to the manufacturer's protocols.

2.7. qRT-PCR analysis

Total RNA in kidney tissues was extracted with the Trizol reagent (Invitrogen, Carlsbad, CA, USA) and treated with DNase I (MBI Fermentas, Lithuania). RNA was reverse transcribed to cDNA with the Revert Aid First Strand cDNA Synthesis Kit (MBI Fermentas, Lithuania) according to the manufacturer's instructions. The total cDNA was used for qRT-PCR with a 7500 real-time PCR System (Applied Biosystems, Carlsbad, California, USA). Specific primers used to amplify specific genes were shown in Table 1. The gene expression was normalized to GAPDH and estimated by 2(−deltadeltaCt) method.

2.8. Terminal deoxynucleotidyl transferase-mediated nick-end labeling (TUNEL) assays

The apoptosis of kidney tissue was determined by the TUNEL assay according to the manufacturer's protocols (Roche Diagnostics, Germany).

2.9. Western blot analysis

Total protein in kidney tissues were extracted with T-PER Tissue Protein Extraction Reagent Kit according to the manufacturer's instructions. Protein concentrations were examined by BCA method. The samples were separated by SDS-PAGE using Tris–HCl Precast Gels, and then transferred onto polyvinylidene difluoride (PVDF) membrane. The membrane was blocked with Tris buffered saline (TBS) containing 3% BSA at room temperature for 2 h, followed by incubation with antibodies to CYP2E1 (Cambridge, UK), p-p65, p-p38, Bax, p-p53 and cleaved caspase 3 at 4 °C overnight. Subsequently, the membrane was washed with PBST and incubated with the secondary antibody conjugated with horseradish peroxidase at room temperature for 1 h. Finally, blots were developed with the ECL through Western Blotting Detection System (Amersham Life Science, UK).

2.10. Statistical analysis

Experimental data were analyzed using SPSS version 19.0 software (SPSS Inc., USA). Differences among the groups were performed with one-way analysis of variance (ANOVA), followed by post-hoc Dunnett's test. Values were expressed as the means ± standard deviation (SD). P-values of <0.05 were considered significant.

3. Results

3.1. Morin ameliorated CP-induced kidney dysfunction

Body weight and relative kidney weight (Fig. 2A and B) were measured at 72 h after CP administration. CP administration reduced body weight and increased kidney weight compared with the control group, but these changes were lightened by morin.

The levels of creatinine and BUN (Fig. 2C and D) were significantly increased by CP treatment compared with the control group, which were decreased by morin in a dose-dependent manner.

3.2. Morin attenuated CP-induced histopathological changes in the kidneys

Histopathological examination of kidney tissues revealed normal kidney architecture in Group I (control group; Fig. 3A) and Group VI (only treated with morin; Fig. 3F). Necrotic epithelial cells, cellular vacuolization and cast formation were observed in Group II (CP injection; Fig. 3B). Compared to CP treated group, morin significantly

Table 1
Primers used in this study.

Primer name	Nucleotide sequence (5′–3′)
TNF-α forward	CTTCTCATTCCTGCTTGTG
TNF-α reverse	ACTTGGTGGTTTGCTACG
IL-1β forward	TTGTGGCTGTGGAGAAG
IL-1β reverse	CATCAGAGGCAAGGAGG
GAPDH forward	AGGTCGGTGTGAACGGATTTG
GAPDH reverse	GGGGTCGTTGATGGCAACA