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Xanthohumol attenuates cisplatin-induced nephrotoxicity through inhibiting NF-κB and activating Nrf2 signaling pathways

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ABSTRACT

Cisplatin is a chemotherapeutic agent that widely used in the treatment of cancer. However, cisplatin has been reported to induce nephrotoxicity by directly inducing inflammatory response and oxidative stress. In this study, we aimed to investigate the protective effects and mechanism of xanthohumol on cisplatin-induced nephrotoxicity. The model of nephrotoxicity was induced by intraperitoneal injection of cisplatin and xanthohumol was given intraperitoneally for three consecutive days. The results showed that xanthohumol significantly attenuated kidney histological changes and serum creatinine and BUN production. The levels of TNF-α, IL-1ß and IL-6 in kidney tissues were suppressed by xanthohumol. The levels of malondialdehyde (MDA) and ROS were suppressed by treatment of xanthohumol. The activities of glutathione (GSH) and superoxide dismutase (SOD) decreased by cisplatin were reversed by xanthohumol. Furthermore, the expression of TLR4 and the activation of NF-κB induced by cisplatin were significantly inhibited by xanthohumol. The expression of Nrf2 and HO-1 were dose-dependently up-regulated by the treatment of xanthohumol. In conclusion, xanthohumol protects against cisplatin-induced nephrotoxicity by ameliorating inflammatory and oxidative responses.

1. Introduction

Cisplatin has been known as one of the most applicable anticancer agent that usually used in the treatment of malignancies [1]. However, studies showed cisplatin had side effects, such as organ toxicity [2]. Nephrotoxicity has been identified as one of the most important side effects induced by cisplatin, which occurred in 20%–30% of patients administration of this drug [3,4]. Till now, the exact mechanism of cisplatin-induced nephrotoxicity has not been fully elucidated. A large body of evidences suggested that inflammation and oxidative stress played critical roles in the development of cisplatin-induced nephrotoxicity [4,5]. Therefore, inhibition of inflammatory and oxidative stress may attenuate cisplatin-induced nephrotoxicity [6]. Many natural herbal compounds have been reported to exhibit anti-inflammatory and anti-oxidative effects [7,8]. These compounds may prevent cisplatin-induced nephrotoxicity and expand the clinical application of cisplatin.

Xanthohumol, a natural prenylated flavonoid isolated from the hop plant (*Humulus lupulus* L.), has been reported to have anti-inflammatory effect [9]. Xanthohumol was found to inhibit LPS-induced acute lung injury in mice [10]. Xanthohumol also suppressed LPS-induced inflammatory cytokine production in BV2 microglial cells [11]. A previous study showed that xanthohumol could inhibit IFN-gamma and LPS-activated macrophages [12]. Studies also showed that xanthohumol had protective effects against carbon tetrachloride-induced acute liver injury [13] and inhibited inflammatory response in ischemia-reperfusion-induced liver injury [14]. However, the protective effects of xanthohumol against cisplatin-induced nephrotoxicity have not been elucidated until now. The purpose of this study was to investigate the protective effects of xanthohumol on cisplatin-induced nephrotoxicity.

2. Materials and methods

2.1. Reagents

Xanthohumol (purity > 98%) was purchased from the National Institutes for Food and Drug Control (Beijing, China). Cisplatin was purchased from Sigma-Aldrich (St. Louis, MO, USA). NF-κB p65, IκBα, and β-actin antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Nrf2, HO-1, and Lamin B antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). MDA, SOD, and GSH kits were purchased from Nanjing jiancheng biological engineering research institute (Nanjing, China). ELISA kits for TNF-α, IL-6

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and IL-1β were obtained from R&D Systems (Minneapolis, MN).

2.2. Model establishment and treatment

C57BL/6 mice (18–22 g) were purchased from the Chinese PLA General Hospital. The mice were given a standard diet and plenty of water. After a five days adaptation period, the mice were randomly divided into six groups and each group contained twelve mice: Control group, Xanthohumol (50 mg/kg) group, Cisplatin group, Cisplatin + Xanthohumol (12.5, 25, 50 mg/kg) groups. All animal experiments were performed according to the Committee of Animal Experimentation of the Chinese PLA General Hospital. The mice of cisplatin group were received cisplatin (20 mg/kg) by intraperitoneal injection. Xanthohumol (12.5, 25, 50 mg/kg) were given by intraperitoneally for three consecutive days. The fourth day after cisplatin treatment, the mice were sacrificed and the kidney tissues and blood were collected for subsequent experiments.

2.3. Histological examination

The mice were sacrificed and the kidney tissues were fixed in 10% buffered formalin for 1 day. The tissues were embedded in paraffin and cut into sections. Then, the sections were stained with H&E staining according to the manufacturer's instruction. The morphological changes of kidney tissues were observed under a light microscope.

2.4. Renal function

The blood was obtained and the serum was collected. The levels of creatinine and BUN in serum were measured using an AutoAnalyzer (Dri-chem 4000i, Fujifilm Co., Tokyo, Japan) according to the manufacturer's instruction.

2.5. Oxidative stress assay

To investigate the oxidative stress of kidney tissues, the levels of MDA, SOD, and GSH in the homogenates of kidney tissues were measured by commercially kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instruction. The level of ROS was measured based on the oxidation of DCFH-DA to DCF as described previously [15].

2.6. Measurement of inflammatory cytokines

The kidney tissues were collected and the levels of inflammatory cytokines TNF-α, IL-1ß and IL-6 in kidney tissues were measured by ELISA kits (R&D System, Minneapolis, MN, USA) according to the manufacturer's instruction.

2.7. Western blot analysis

Nuclear and Cytoplasmic Proteins were extracted from the kidney tissues using NE-PER Mammalian Protein Extraction Reagent (Thermo Scientific; IL, USA). Protein concentration was determined by BCA protein assay kit. Equal amounts of proteins were separated 12% SDS-PAGE and blotted onto PVDF membranes. After blockade of non-specific binding sites used by 5% skim milk for 2 h at room temperature, the membranes were washed three times with TBST. Then the membranes were probed with primary antibodies (1:1000 dilutions in TBST, Nrf2, HO-1, p-NF-κB p65, NF-κB p65, p-IκBα, IκBα, and β-actin) at 4 °C overnight. The next day, the membranes were washed 3 times. Followed by incubation with secondary antibody served a peroxidase-conjugated donkey anti-rabbit IgG (1: 5000 dilutions in TBST) with incubation for 2 h at room temperature. After washing three times with TBST, proteins were visualized with ECL-chemiluminescent kit.

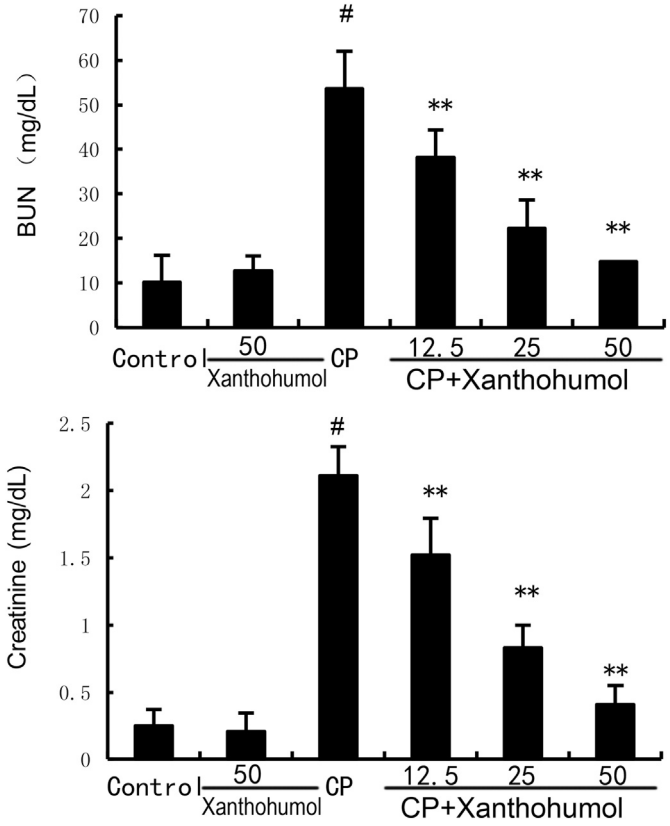

Fig. 1. Effects of xanthohumol on BUN and creatinine levels in serum. The values presented are the mean ± SEM of three parallel measurements. $p\# < 0.01$ vs. control group, $p^* < 0.05$, $p^{**} < 0.01$ vs. CP group.

2.8. Statistical analysis

All data are shown as mean ± SEM. One-way ANOVA and Tukey–Kramer multiple comparison test were used to compare the differences between groups. $p < 0.05$ were considered significant.

3. Results

3.1. Xanthohumol inhibits cisplatin-induced BUN and creatinine production

In this study, we detected the effects of xanthohumol on kidney function by measuring serum BUN and creatinine. As shown in Fig. 1, compared with the control group, the mice of cisplatin-treated group exhibited marked increases in the levels of BUN and creatinine in serum. Compared with those in cisplatin-treated group, xanthohumol (12.5, 25, 50 mg/kg) groups significantly attenuated the levels of BUN and creatinine in serum (Fig. 1).

3.2. Xanthohumol attenuates cisplatin-induced renal tissue injury

Kidney histopathologic changes were detected in this study to investigate the effects of xanthohumol on renal tissue injury. As shown in Fig. 2A, the kidney tissues of control group showed normal morphology. The kidney tissues of cisplatin group showed significant renal tubular vacuolar changes, including tubular epithelial cells sloughing, loss of brush border and epithelial cell nuclei, and infiltration of inflammatory cells (Fig. 2B). However, these changes were dose-dependently inhibited by xanthohumol (Fig. 2C, D, E).

3.3. Effects of xanthohumol on cisplatin-induced oxidative stress

To investigate the effects of xanthohumol on cisplatin-induced