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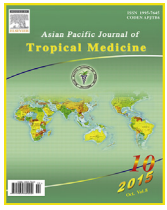

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Therapeutic effect and mechanism of breviscapine on cisplatin-induced nephrotoxicity in mice

Xiao-Yu Lou[1,2], Jing-Liang Cheng[1*], Bo Zhang[3]

[1]*Department of Magnetic Resonance Imaging, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450003, Henan, China*

[2]*Department of Magnetic Resonance Imaging, The First Affiliated Hospital of Luohe Medical College, Luohe 462002, Henan, China*

[3]*Department of Ultrasound in Medicine, East Hospital, Tongji University School of Medicine, Shanghai 200120, China*

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ABSTRACT

Objective: To observe the protective effect of breviscapine on mice with cisplatin-induced nephrotoxicity.

Methods: Mice were given a single injection of cisplatin (8 mg/kg, *i.p.*); then, breviscapine was given to mice at 25 mg/kg and 50 mg/kg doses, respectively, once a day for seven days. Renal tissue structure was observed after animals were sacrificed. Blood urea nitrogen (BUN), serum creatinine (Scr), lipid peroxide (MDA) and superoxide dismutase (SOD) serum levels were detected; and MDA, glutathione peroxidase, and SOD levels in the renal cortex were detected.

Results: Compared with the blank control group (BCG), the kidney pathological damage of mice in the model control group (MCG) was more severe. After applying different doses of breviscapine, different degrees of renal injury improvement appeared. Compared with the BCG, the serum levels of Scr and BUN in the MCG increased to (89.92 ± 6.78) μmoL/Land (15.32 ± 4.53) mmoL/L. The differences were statistical significant ($P < 0.01$). Compared with the MCG, the serum levels of Scr and BUN in the Bre low-dose groups and Bre high-dose groups decreased significantly ($P < 0.05$). Compared with the BCG, the MDA levels in serum and in the renal cortex in the MCG significantly increased, while the SOD levels significantly decreased. Both the differences were statistically significant ($P < 0.01$). In the Bre low-dose groups and Bre high-dose groups, MDA levels in serum and in the renal cortex significantly decreased, while SOD and glutathione peroxidase levels in the renal cortex significantly increased, compared with the MCG; and the differences were statistically significant ($P < 0.05$).

Conclusions: Breviscapine can reduce cisplatin-induced renal toxicity in mice and it's possible through inhibition of renal tubule cell lipid peroxidation and reduces the nephrotoxicity of cisplatin.

1. Introduction

Cisplatin is a common cycle nonspecific clinical platinum anticancer drug that has achieved a satisfactory antineoplastic effect in the treatment of a variety of solid tumors [1,2]. Cisplatin is involved in approximately 80% of antineoplastic combined chemotherapy protocols. Since the antineoplastic effect of cisplatin is dose dependent, high doses would lead to renal injury, gastrointestinal reactions, ototoxicity and peripheral neuropathy [3,4]. The high aggregation, hypermetabolism and discharge characteristics of cisplatin in kidneys may cause acute or chronic renal injury; and although hydration treatment can prevent the nephrotoxicity of cisplatin to some extent, renal dysfunction appears in approximately 25%–30% of patients after reaching a dosage of 80 mg/cm^2 [5]. Renal toxicity is a main obstacle in the clinical usage of cisplatin, and determining how to control renal toxicity while retaining the antineoplastic effect of cisplatin is the main focus in clinical researches. Breviscapine (Bre) is a kind of flavonoid extracted from the compositae plant, erigeron breviscapus; which has shown anti-microbial, anti-inflammatory, free radical-eliminating and antineoplastic effects [6,7]. A study has reported its curative effect in the treatment of diabetic and hypertensive nephropathy [8], but whether it has a protective

*Corresponding author: Jing-Liang Cheng, Department of Magnetic Resonance Imaging, the First Affiliated Hospital of Zhengzhou University, Zhengzhou 450003, Henan, China.
Tel: +86 371 67966331, +86 13603863860 (mobile)
E-mail: zhy6290@163.com

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effect on cisplatin-induced renal nephropathy remains unclear. In this study, a cisplatin-induced Kunming mice nephropathy model was established; then, pathological sections and blood biochemistry were analyzed to investigate the effect of Bre on renal toxicity, and explore its mechanism.

2. Materials and methods

2.1. Drugs and reagents

Bre lyophilized powder (50 mg/bottle) was purchased from Hunan Hangseng Pharmaceutical Co., Ltd. (20100403). Cisplatin lyophilized powder (20 mg/bottle) was purchased from Shandong Qilu Pharmaceutical Product (908027 CF). Creatinine (Scr) test kit, blood urea nitrogen (BUN) test kit, malondialdehyde (MDA) detection kit, and superoxide dismutase (SOD) detection kit were supplied by Nanjing Jiancheng Biological Engineering Co., Ltd. Other reagents were all pure analysis.

2.2. Animals and equipment

Equal numbers of male and female Kunming mice, weighting 18–22 g, were purchased from the Medical Experimental Animal Center of Henan Province; and were used in the experiment. The 752N ultraviolet visible spectrophotometer was provided by Shanghai Precision and Scientific Instrument Co., Ltd. The TL80-1 medical centrifuge was obtained from Jiangsu JiangyanTianli Equipment Co., Ltd. The AU2700 automatic biochemical analyzer and BX40 optical microscope were obtained from Olympus, Japan.

2.3. Groups and drugs

Kunming mice were randomly divided into four groups according to weight with 12 mice in each group: (1) blank control group (BCG), mice were routinely fed and lavaged with 0.5% CMC-Na solution; (2) model control group (MCG), a cisplatin-induced renal injury in mice model was established by intraperitoneally injecting cisplatin lyophilized powder diluted with 8 mg/kg of saline water, and mice were lavaged with 0.5% CMC-Na solution; (3) Bre low-dose group (BLDG), after a nephropathy model was established, mice were lavaged with a Bre mixed suspension (0.5% CMC-Na) at 25 mg/kg; (4) Bre high-dose group (BHDG), after a nephropathy model was established, mice were lavaged by a Bre mixed suspension (0.5% CMC-Na) at 50 mg/kg. After establishing a nephropathy model, Bre was administered to all animals once a day for seven days, with a drug delivery volume of 0.1 mL/10 g.

2.4. Observation of kidney morphology

On the eighth day, two mice were randomly selected from each group, and sacrificed by dislocation. Kidneys were taken out by caesarean section. Kidney tissues were fixed with 10% formalin, embedded in paraffin, sliced in a paraffin slicing machine, and H&E staining was performed. Shape and structure of kidney tissues were observed under a light microscope.

2.5. Preparation and determination of samples

After administering ether anesthesia, blood was collected from the angular vein of mice and placed into a tube with heparin anticoagulant, centrifuged at 3000 r/min for 10 min, and stored in a refrigerator at −4 °C. Mice were sacrificed, kidneys were quickly removed and placed in a refrigerator at −40 °C, frozen kidneys were taken out, small renal cortical tissues were chipped in the ice bath, 0.1 moL/L of PBS solution (pH = 7.4, 4 °C) was added, 10% of the renal cortical tissue homogenate was made by grinding, centrifuged at 3000 r/min for 10 min, and the liquid supernatant was placed in a refrigerator at −4 °C.Scr and BUN were determined using an automatic analyzer. MDA, SOD and glutathione peroxidase (GSH-Px) were analyzed according to the kit's instructions.

2.6. Statistical analysis

Results were expressed as mean ± SEM. SPSS 11.0 was used for statistical analysis. One-way analysis of variance and *t*-test comparison were applied in the statistical analysis. $P < 0.05$ was considered significant.

3. Results

3.1. Cisplatin-induced kidney structural changes in mice

H&E staining results revealed that kidney structure was normal and renal tubular and glomerular structures were clear in the BCG. Compared with the BCG, mice in the MCG had severe renal pathological injury, renal tubular epithelial cell swelling occurred, cell vacuolization, necrosis, and shedding appeared, fibrous tissue proliferated in the renal interstitium, infiltration of a large number of inflammatory cells occurred, and parts of the brush-border were damaged. After applying different doses of Bre, renal injury improved in different degrees. In the BLDG, renal tubules were slightly swollen, and necrosis occurred in some renal tubules. The fibrous hyperplasia in renal interstitium was not obvious, with a small amount of inflammatory cell infiltration, and some parts of the brush-border fell off. In the BHDG, renal tissue structure was basically normal, and only the renal tubules were slightly swollen. The renal interstitium was normal (As shown by the arrows in Figure 1).

3.2. Effect of Bre on GSH-Px serum activity in cisplatin-induced renal injury mice

GSH-Px activity decreased in the MCG, compared with the BCG; and the difference was statistically significant ($P < 0.05$). GSH-Px activity in the BLD and BHDGs remained much lower than the BCG, but was significantly improved in the MCG; and the difference was statistically significant ($P < 0.05$) (Figure 2).

3.3. Effect of Bre on Scr and BUN serum levels in cisplatin-induced renal injury in mice

Scr and BUN serum levels significantly improved in the MCG ($P < 0.01$), compared with the BCG; while Scr and BUN serum levels in the BLD and BHDGs significantly increased, compared with the MCG ($P < 0.05$). These results indicate that cisplatin can induce renal injury, while Bre can alleviate renal injury in a dose dependent manner (Table 1).