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Differential effects of dihydropyridine calcium antagonists on doxorubicin-induced nephrotoxicity in rats

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Abstract

The aim of this study was to compare the roles of dihydropyridine calcium antagonists nifedipine, nitrendipine, amlodipine on doxorubicin (DXR)-induced nephrotoxicity in rats using biochemical, histopathological and immunohistochemical approaches. Male Sprague–Dawley rats were randomly divided into five experimental groups: control; DXR; DXR + nifedipine (15 mg/kg); DXR + nitrendipine (10 mg/kg); DXR + amlodipine (5 mg/kg). Results showed that treatment with DXR alone caused significant changes in the levels of urinary protein, serum creatinine (SCr), and blood urea nitrogen (BUN). Co-administration with amlodipine effectively reversed the effect of DXR on these parameters. In contrast, nifedipine and nitrendipine either had no effect or worsened DXR induced changes in the levels of urinary protein, SCr and BUN. Furthermore, DXR treatment caused significant increases in the levels of malondialdehyde (MDA), nitric oxide (NO), nitric oxide synthase (NOS) and significant decreases in the levels of reduced glutathione (GSH), glutathione-*S*-transferase (GST), and superoxide dismutase (SOD). These effects were significantly reduced by co-administration with amlodipine but not affected by nifedipine and worsened by nitrendipine. In addition to the biochemical changes, histopathological studies showed that DXR caused significant structural damages in the kidneys. Glomerular cell apoptosis, a decrease in *Bcl-2* expression and an increase in *Bax* expression were observed in all rats treated with DXR. Co-administration with amlodipine effectively reversed the effect of DXR while nifedipine and nitrendipine had no effect. In conclusion, this study clearly indicated that amlodipine protected against DXR-induced nephrotoxicity while nifedipine and nitrendipine had no effect.

Keywords: Nifedipine; Nitrendipine; Amlodipine; Dihydropyridine; Doxorubicin; Nephrotoxicity; Antioxidants; Apoptosis

1. Introduction

Since the introduction of doxorubicin (DXR) for the treatment of cancer in 1969, this chemical has demonstrated high antitumor efficacy. However, its use in chemotherapy has been limited largely due to its diverse toxicities, including cardiac, renal, hematological and testicular toxicity (Gillick et al., 2002; Yilmaz et al., 2006). DXR-induced changes in the kidneys of rats include glomerular capillary permeability increases and glomerular atrophy (Saad et al., 2001). Although the exact mechanism of DXR-induced nephrotoxicity remains unknown, it has been believed to be mediated through free radical formation, iron-dependent oxidative damage of biological macromolecules, and membrane lipid peroxidation (Saad et al., 2001; Pritsos and Ma, 2000). Two different pathways of free radical formation

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by DXR have been described. The first implicates the formation of a semiquinone free radical by the action of several NADPH-dependent reductases that produce a one-electron reduction of DXR to the corresponding DXR semiquinone. In the presence of oxygen, redox cycling of DXR-derived quinine–semiquinone yields superoxide radicals ($O_2^{\bullet -}$). In the second pathway, DXR free radicals are produced by a non-enzymatic mechanism that involves reactions with iron. Iron–DXR complex can reduce oxygen to H_2O_2 and other active oxygen species, which cause oxidative damage of a variety of tissues including the kidneys (Singal et al., 2000; De Beer et al., 2001).

Calcium antagonists represent an important group of drugs for the treatment of a variety of cardiovascular diseases. Among the clinically used calcium antagonists, dihydropyridines (DHP-CAs) are the most frequently used. Apart from their antihypertensive actions, they are beneficial to the kidney and are widely used in supplemental therapy of kidney diseases (Tobe, 2003; Bakris et al., 2004). However, it is not known whether or not DHP-CAs have any protective effect on drug-induced nephrotoxicity. Treatment with nifedipine (10 and 20 mg/kg) significantly improved renal dysfunction, tissue and urinary total nitric oxide levels, and renal oxidative stress and prevented the alterations in renal morphology induced by cyclosporine. These results clearly demonstrate that nifedipine is beneficial as a protective agent against nephrotoxicity induced by cyclosporine (Chander and Chopra, 2005). Pretreatment with a long acting calcium antagonist amlodipine has no effect on the course of urinary alpha-glutathione-*S*-transferase excretion and creatinine clearance induced by radiocontrast (Arici et al., 2003). However, it protected the renal tissue from nephrotoxicity induced by diatrizoate (Duan et al., 2000). The present study was therefore designed to evaluate the diverse effects of different DHP-CAs on DXR-induced nephrotoxicity in rats.

2. Materials and methods

2.1. Chemicals

DXR was purchased from Shangdong Dezhou Pharmaceutical Factory (Dezhou, China). Nifedipine, nitrendipine and amlodipine were purchased from Shanxi Linfen Pharmaceutical Factory (Linfen, China), Zhengzhou Sanjiusande Pharmaceutical Ltd. (Zhengzhou, China) and Pfizer Pharmaceuticals Ltd., China (Dalian, China), respectively. Apoptosis Measurement Kit was obtained from Boehringer Mannhelm Corp. (Mannheim, Germany). The *bcl-2* monoclonal antibody and *Bax* polyclonal antibodies and biotinylated goat anti-rabbit IgG Kit were purchased from Beijing Zhongshan Biotechnology Co. Ltd. (Beijing, China). All other chemicals were obtained from Sigma (St. Louis, MO, USA).

2.2. Experimental procedures

All studies involving animals were approved by the Animal Care Committee of Wuhan University. In this investigation, 35 healthy male Sprague–Dawley rats (8-week-old weighing 180 ± 20 g) were used. The animals were obtained from Wuhan University Experimental Research Centre, Wuhan, China. The animals were housed in standard cages at room temperature (20–24 °C) and regular light cycle (12 light/12 dark) with food and water *ad libitum*. The rats were divided into five groups of seven animals each. Control group: rats were injected intravenously (i.v.) through a tail vein with 0.9% NaCl (10 ml/kg) and followed by intragastric gavage daily with distilled water (10 ml/kg) for 30 days. DXR group: rats were injected i.v. a single dose of DXR (6.5 mg/kg) through a tail vein and followed by intragastric gavage daily with distilled water (10 ml/kg) for 30 days. DXR plus nifedipine group: rats were injected i.v. a single dose of DXR (6.5 mg/kg) through a tail vein and followed by intragastric gavage daily with nifedipine (15 mg/kg) for 30 days. DXR plus nitrendipine group: rats were injected i.v. a single dose of DXR (6.5 mg/kg) through a tail vein and followed by intragastric gavage daily with nifedipine (10 mg/kg) for 30 days. DXR plus amlodipine group: rats were injected i.v. a single dose of DXR (6.5 mg/kg) through a tail vein and followed by intragastric gavage daily with amlodipine (5 mg/kg) for 30 days.

Twenty-four-hour urines were collected at the 10th, 20th and 30th day after administration of nifedipine, nitrendipine, and amlodipine to measure the content of urinary protein. The animals in all groups were anesthetized with 45 mg/kg sodium pentobarbital and were sacrificed 4 h after the last administration. Blood samples were collected to measure SCr and BUN. The samples were centrifuged at $200 \times g$ for 5 min at +4 °C. Kidneys were removed rapidly, excised and sectioned for histological analysis. The remaining kidney tissues were homogenized in Tris–HCl buffer (0.05 mol/L Tris–HCl, 1.15% KCl, pH 7.4), using a Polytron homogeniser. The homogenate was centrifuged at $18{,}000 \times g$ (+4 °C) for 30 min, the supernatant was utilized for biochemical analysis.

2.3. Biochemical assays

Urinary protein content was measured according to the sulfosalicylic acid colorimetric method (Salant and Ybulsky, 1988). BUN and SCr concentrations were measured using diacetyl monoxime and basic picric acid as substrates, respectively. The concentrations of MDA were determined according to the method based on the reaction with thiobarbituric acid (Ohkawa et al., 1979). SOD activity was assayed using pyrogallol as a substrate (Marklund and Marklund, 1974). GSH level was measured colorimetrically as protein-free sulfhydryl content using DTNB Ellman reagent (Beutler et al., 1963). GST activity was determined spectrophotometrically using CDNB