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Amelioration of cisplatin-induced nephrotoxicity by pravastatin in mice

Yi An [a], Hui Xin [a], Wei Yan [b], Xiaoxu Zhou [b,*]

[a] Department of Cardiology, The Affiliated Hospital of Qingdao University Medical College, China
[b] Department of Cardiology, The First Affiliated Hospital of Harbin Medical University, China

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ABSTRACT

This study investigated the protective effects of pravastatin against cisplatin-induced nephrotoxicity and the possible mechanisms in mice. Pravastatin showed significant protection as evidenced by the decrease of elevated serum creatinine (CRE) and blood urea nitrogen (BUN), and improvement of histopathological injury induced by cisplatin. The formation of kidney malondialdehyde (MDA) with a concomitant reduction of reduced glutathione (GSH) were inhibited by pravastatin, while the activities of kidney superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-px) were increased. The over expressions of kidney induced nitric oxide synthase (iNOS) and nitrotyrosine (3-NT) were suppressed by pravastatin. Pravastatin suppressed cisplatin-induced p38 mitogen-activated protein kinase (MAPK) activation in the kidney of mice. These results suggest that pravastatin pre-administration can prevent the nephrotoxicity induced by cisplatin. Pravastatin may exert the protective effect via inhibiting oxidative and nitrosative stress.

1. Introduction

Cisplatin (cis-diamminedichloroplatinum) is an effective anticancer drug for the treatment of several human solid tumors (Lebwohl and Canetta, 1998). Despite the encouraging clinical response to cisplatin-based chemotherapy, there are many occasions in which it is difficult to continue the administration of the drug due to its nephrotoxicity (Madias and Marrington, 1978; Taguchi et al., 2005). Primary target of cisplatin in kidney is the proximal tubules, where it accumulates and promotes cellular damage (Kawai et al., 2005). Cisplatin-induced nephrotoxicity is associated with increased renal vascular resistance and morphological damage to the intracellular organelles, including cellular necrosis, loss of microvilli, changes in the number and size of lysosomes, and mitochondrial vacuolization. The cisplatin-induced alterations in kidney functions are characterized by signs of injury, including glutathione status and lipid peroxidation (Daugaard, 1990).

Cellular and molecular mechanisms responsible for cisplatin-induced nephrotoxicity are not well understood, but there is evidence that the formation of reactive oxygen species (ROS) is involved in producing kidney damage (Matsushima et al., 1998). Cisplatin is taken up preferentially and accumulates in the human kidney cells (Stewart et al., 1982), resulting in the enhanced production of ROS and the decrease in the antioxidant enzymes (Weijl et al., 1997; Sadzuka et al., 1992). Excessive ROS generation caused by cisplatin may overwhelm the natural antioxidant defenses of the kidney cell and lead to lipid peroxidation (LPO) and delayed-onset kidney injury (Vermeulen and Baldew, 1992). Cisplatin also increased kidney iNOS activity and formation of NO which reacts with $O2\cdot-$ to form peroxinitrite, a toxic agent to the cellular components (Srivastava et al., 1996). Exposure to reactive nitrogen species (RNS) may induce the LPO in cell membranes, which in turn may generate species that damage cell proteins and promote their degradation (Eiserich et al., 1998).

Pravastatin, a hydrophilic inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, has lipid-lowering activity (Corsini et al., 1995). Recently, HMG-CoA reductase inhibitors have been postulated to have pleiotropic effects, such as restoration of endothelial function, stabilization of atherosclerotic plaques, reduction of oxidative stress, anti-inflammatory actions, inhibition of thrombosis, suppression of smooth muscle cell proliferation, improvement of insulin sensitivity, and enhancement of vasculogenesis (Simins, 2000). Although pravastatin is validated to inhibit oxidative stress, not only the effects and mechanisms of pravastatin acting on cisplatin-induced nephrotoxicity, but also the effect of pravastatin on cisplatin-induced nitrosative stress are still intangible.

Oxidative stress can activate the intracellular MAPK pathway and the increased MAPKs are thought to be important in the

Abbreviations: 3-NT, 3-nitrotyrosine; BUN, blood urea nitrogen; CAT, catalase; Cisplatin, cis-diamminedichloroplatinum; CRE, creatinine; GSH, glutathione; GSH-px, glutathione peroxidase; H&E, hematoxylin–eosin; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; iNOS, induced nitric oxide synthase; LPO, lipid peroxidation; MDA, malondialdehyde; PBS, phosphate buffered saline; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase

* Corresponding author at: 23 Youzheng Str., Nangang District, Harbin, China. Tel.: +86 13836016424; fax: +86 0532 8291 3126.
E-mail address: zhouxiaoxu@yahoo.com.cn (X. Zhou).

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pathogenesis of cisplatin-induced renal injury (Arany et al., 2004. Ramesh and Reeves, 2005). However, the relationship between the protective effect of pravastatin and MAPKs in cisplatin-induced nephrotoxicity of mice is unclear.

The purpose of the present study was to assess whether pravastatin prevents cisplatin-induced nephrotoxicity in mice and the related mechanisms. We evaluated if the protective effect of pravastatin treatment on cisplatin-induced nephrotoxicity is associated with the activation of MAPKs in mice kidney.

2. Materials and methods

2.1. Materials

Pravastatin was kindly provided by Sankyo Co. Ltd. (Japan) (purity > 99%). Cisplatin were purchased from Aote Chemical Inc., Ltd. (Zibo, China). BUN and CRE kits were purchased from BHKT Chemical Reagent Co., Ltd (Beijing, China). Anti-3-NT and anti-iNOS antibodies used in ours study were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Twenty-seven male ICR mice weighing 20–22 g were purchased from Beijing Vital River Laboratory Animal Co. Ltd.

2.2. Animal treatment

Mice were maintained in a temperature 22 ± 2 °C and relative humidity 55 ± 15%-controlled room with a 12-h light and dark cycle and were allowed food and tap water ad libitum. Mice were maintained in these facilities for 3 days before the experiment. The ICR mice were randomly divided into the following 3 groups, each group containing 9 mice: control group, cisplatin group and pravastatin plus cisplatin group. The mice in pravastatin plus cisplatin group received pravastatin (80 mg/kg) in 0.05% carboxymethylcellulose (CMC) by oral gavage. Two hours after treatment with the pravastatin, cisplatin (5 mg/kg) in phosphate buffered saline (PBS) was injected intraperitoneally. The pravastatin and cisplatin were administered once daily over a 5-day period. Control mice received 0.05%CMC and PBS. Sixteen hours after the last cisplatin injection, the mice were killed. Blood was sampled from ophthalmic veins to obtain serum after centrifugation. Serum and kidney tissue samples were stored at −80 °C before assay. All the experiments were approved by the Committee on the Care and Use of Animals in Research at the Animal Care and Use Committee, of Qingdao University Medical College.

2.3. Histopathological observation

Kidney tissue was fixed in 10% neutral buffered formalin and embedded into paraffin blocks. Histopathological evaluation was done on 5 μm-thick, hematoxylin–eosin (H&E) stained sections.

2.4. Biochemical assay

BUN and CRE were determined by spectrophotometry using commercially available kits.

2.5. Determination of renal MDA, GSH, GSH-px, SOD and CAT

Kidney tissue was homogenized with 10 volumes of ice-cold 1.15% KCl buffer containing 0.4 mM PMSF and was centrifuged at 2000 rpm for 10 min (4 °C). MDA content was quantified in the form of thiobarbituric acid reactive substances at 532 nm. 1,1,3,3-Tetramethoxypropane was used to establish a standard curve. GSH was estimated colorimetrically at 412 nm after its complex formation with dithio-bis-nitrobenzoic acid. The remaining homogenate was centrifuged at 4000 rpm for 30 min at 4 °C for the assay of antioxidant enzymes. GSH-px activity was determined spectrophotometrically by measuring the oxidized glutathione formed in the glutathione peroxidase reaction by coupling it to the oxidation of reduced form of nicotinamide-adenine dinucleotide phosphate via glutathione reductase. Total (Cu–Zn and Mn) SOD activity was determined according to Sun et al. (1988). The determination of CAT activity was based on the reaction of the enzyme with methanol in the presence of an optimal concentration of hydrogen peroxide. The formaldehyde produced was measured with Purpald as a chromogen at 540 nm. The protein content of liver homogenate was measured according to Lowry et al. (1951).

2.6. Western blotting analysis

Kidney tissue was prepared by homogenation in lysis buffer (50 mM Tris, pH 7.5, 150 mM sodium chloride, 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, 1% Nonidet P-40, 50 mM sodium fluoride, 10 μg/ml proteinase inhibitors mixture, 10% glycerol) at 4 °C, followed by the centrifugation at 16,000 rpm at 4 °C for 10 min. After quantification of protein concentrations, the supernatants were mixed with Laemmli loading buffer, boiled for 4 min, and then submitted to Western blot analysis. Membranes were blotted against primary antibodies (anti-3-NT and anti-iNOS antibodies) at 4 °C for 16 h, washed with 0.1% (vol/vol) Tween 20 in TBS (pH 7.4), and incubated with horseradish peroxidase-conjugated secondary antibodies for 45 min. Protein bands were visualized by the enhanced chemiluminescence reaction method (Applygen Technologies Inc., Beijing). The densities of bands were measured by gelpro32 software program.

2.7. Statistical analysis

Experimental values are expressed as mean ± SD. Comparison of mean values between groups was performed by one way-analysis of variance (one way-ANOVA) followed by post-hoc Tukey test. $P < 0.05$ and $P < 0.01$ were considered to be significant.

3. Results

3.1. Pravastatin improved cisplatin-induced nephrotoxicity

Cisplatin induced a nephrotoxicity, as judged by the elevated serum CRE, BUN and histopathological changes including tubular necrosis, vacuolation and desquamation of epithelial cells in renal tubules and tubular cast (looking at Fig. 1 picture C). Pre-treatment with pravastatin (80 mg/kg) resulted in a significant inhibition on the increasing of serum CRE and BUN, respectively (Table 1). Pre-treatment with pravastatin dramatically improved the cisplatin nephrotoxicity, and less histological damage was observed in renal tubules (Fig. 1).

3.2. Pravstatin inhibited cisplatin-induced oxidative stress

Kidney MDA level, as a maker of lipid peroxidation, was significantly increased in mice treated with cisplatin alone, while GSH was considerably decreased. In addition, the activities of kidney antioxidant enzymes including CAT, SOD and GSH-px were also decreased in cisplatin-treated mice. Pravastatin