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Telmisartan ameliorates cisplatin-induced nephrotoxicity by inhibiting MAPK mediated inflammation and apoptosis

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ABSTRACT

Nephrotoxicity is a major adverse effect of the widely used anticancer drug cisplatin. Oxidative stress, inflammation and apoptosis are implicated in the pathophysiology of cisplatin-induced acute renal injury. Moreover, cisplatin activates many signal transduction pathways involved in cell injury and death, particularly mitogen activated protein kinase (MAPK) pathway. With this background, we aimed to investigate the protective effect of telmisartan, a widely used antihypertensive drug, in cisplatin-induced nephrotoxicity model in rats. To accomplish this, male albino wistar rats (150–200 g) were divided into 6 groups: Normal, cisplatin-control, telmisartan (2.5, 5 and 10 mg/kg) and telmisartan *per se* treatment groups. Normal saline or telmisartan was administered orally to rats for 10 days and cisplatin was given on 7th day (8 mg/kg; i.p.) to induce nephrotoxicity. On 10th day, rats were killed and both the kidneys were harvested for biochemical, histopathological and molecular studies. Cisplatin injected rats showed depressed renal function, altered proxidant–antioxidant balance and acute tubular necrosis which was significantly normalized by telmisartan co-treatment. Furthermore, cisplatin administration activated MAPK pathway that caused tubular inflammation and apoptosis in rats. Telmisartan treatment significantly prevented MAPK mediated inflammation and apoptosis. Among the three doses studied telmisartan at 10 mg/kg dose showed maximum nephroprotective effect which could be due to maintenance of cellular redox status and inhibition of MAPK activation.

1. Introduction

Cisplatin (*cis*-diamminedichloroplatinum) is one of the most effective anticancer drugs used for the treatment of solid cancers of head, neck, testicular, ovarian, cervical and non-small cell lung carcinoma. However, it is associated with dose-limiting nephrotoxicity that occurs in approximately one third of the patients. Cisplatin primarily accumulates in proximal straight and distal convoluted tubules causing cellular damage and impairment of renal function (Terada et al., 2013). Various approaches attempting to limit this side effect have failed and the only treatment available at present is to hydrate the patients with saline and induce diuresis; nevertheless, the effect is only partial and still over a quarter of patients experience renal problems or insufficiency. Thus, it is necessary to investigate newer measures for preventing this dose-limiting side effect of cisplatin, especially when it has to be given at high tumoricidal doses for a long duration.

Multiple mechanisms have been implicated in the pathophysiology of cisplatin-induced renal injury such as oxidative stress, apoptosis and inflammation. Of late, there is growing evidence that members of mitogen activated protein kinases (MAPKs) family; such as extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 are activated after cisplatin administration and contribute to renal cell death (Pabla and Dong, 2008). Nowak demonstrated that cisplatin induced phosphorylation and accumulation of ERK1/2 in the mitochondria of renal proximal tubular cells. Further, administration of ERK1/2 pharmacological inhibitors – PD98059 and U1026 – prevented ERK1/2 mediated cytochrome *c* release, caspase-3 activation and translocation of Bax from cytoplasm to mitochondria, and attenuated cisplatin-induced mitochondrial dysfunction (Nowak, 2002). The role of p38 in nephrotoxicity was highlighted by Francescato et al. They showed that blockade of p38 MAPK activation decreased cisplatin mediated inflammation, oxidative stress and apoptotic cell death in kidney (Francescato et al., 2009). However, the role of JNK in cisplatin-induced renal injury is not well characterized but a recent work by Kim et al. (2014), demonstrated that activation of JNK (phospho-JNK) deteriorated renal function, increased tubular inflammation and apoptosis after cisplatin

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administration, suggesting a mechanistic role of JNK in acute kidney injury. Hence, these observations by various workers suggest that MAPK pathway can be used as a potential target to explore newer therapeutic interventions to curtail cisplatin-induced nephrotoxicity.

Telmisartan is a potent, long-acting, nonpeptide angiotensin II type-1 (AT1) receptor antagonist that has shown anti-inflammatory, antioxidant, anti-apoptotic and nephroprotective effects (Fouad et al., 2010). Many preclinical studies have demonstrated its protective role in doxorubicin-induced nephrotoxicity (Ibrahim et al., 2009), partially nephrectomized rats (Tsunenari et al., 2007), X-ray contrast media induced nephrotoxicity (Duan et al., 2009), renal ischemia–reperfusion injury (Fouad et al., 2010), cadmium-induced nephrotoxicity (Fouad and Jresat, 2011; Ahmed, 2013), and diabetic nephropathy (Ohmura et al., 2012). Also, few clinical studies have confirmed its protective effect against diabetic nephropathy (Barnett, 2005) and hypertensive non-diabetic nephropathy (Aranda et al., 2005). However, there is no study addressing its role in cisplatin-induced acute renal injury. Hence, the present experimental study was conducted to evaluate its renoprotective potential in cisplatin-induced nephrotoxicity and further to investigate whether MAPK pathway mediates this nephroprotection.

2. Material and methods

2.1. Animals

The study was carried out on 36 male albino wistar rats, each weighing 150–200 g. The current study was approved by Institutional Animal Ethics Committee of All India Institute of Medical Sciences, New Delhi, India, (IAEC No. 715/13), and all experimental procedures were conducted in accordance to Indian National Science Academy Guidelines for Care and Use of Animals in Scientific research. The animals were housed in standard laboratory conditions at a constant temperature (25 ± 2 °C) with a relative humidity of 60 ± 5% under a 12 h light/dark cycle. They had free access to standard diet (Ashirwad Industries Ltd., Chandigarh, India) and tap water *ad libitum*.

2.2. Chemicals

Telmisartan and Cisplatin were purchased from Ranbaxy Laboratories Limited, India and Pfizer Products India Pvt. Ltd., India respectively. Blood urea nitrogen (BUN) and serum creatinine kits were obtained from Transasia Bio-Medicals Ltd., India. Terminal deoxynucleotide transferase dUTP nick end labeling (TUNEL) assay kit (ApoBrdu DNA fragmentation assay kit) and TNF-α ELISA kit was obtained from Biovision Inc. California and Diaclone Tepnel Company, UK respectively. Primary antibodies for caspase-3, ERK1/2, phospho-ERK1/2 (p-ERK1/2), JNK and phospho-JNK (p-JNK) were purchased from cell signaling technology, USA while Bcl-2, Bax and p-38 primary antibodies were obtained from Abcam, UK. Primary antibody for phospho-p38 (p-p38) was procured from santa cruz biotechnology, USA. Secondary antibodies were procured from Merck specialties Pvt. Ltd., India. All other chemicals used were of analytical grade.

2.3. Experimental design

The rats were randomly divided into 6 experimental groups. Each group consisted of 6 rats.

Group 1 (Normal): normal saline (3 ml/kg/day; p.o.) was administered to rats for a period of 10 days.

Group 2 (Cisplatin-control): normal saline was administered to rats for a period of 10 days and on 7th day, a single injection of Cisplatin (8 mg/kg; i.p.) was given.

Group 3–5 (Telmisartan 2.5, 5, 10+Cisplatin): Telmisartan (2.5, 5 and 10 mg/kg/day; p.o.) was administered to rats for a period of 10 days and on 7th day, a single injection of cisplatin (8 mg/kg; i.p.) was given.

Group 6 (Telmisartan per se): Telmisartan (10 mg/kg/day; p.o.) was administered to rats for a period of 10 days.

On the 10th day, animals were anaesthetized with pentobarbitone sodium (60 mg/kg; i.p.), blood was collected and then centrifuged at 1860*g* to separate the serum and stored at −20 °C for estimating BUN, serum creatinine and TNF-α levels. Meanwhile, both the kidneys were harvested; one of the kidneys was snap frozen in liquid nitrogen and stored at −80 °C for biochemical and western blot analysis. Other kidney was immediately kept in 10% neutral buffered formalin, embedded in paraffin and used for histopathological, immunohistochemistry (IHC) and TUNEL assay.

2.4. Measurement of kidney function test

Serum creatinine and BUN levels were measured using commercially available kits as per manufacturer's instructions.

2.5. Biochemical estimation

A 10% tissue homogenate was prepared in ice-chilled phosphate buffer (0.1 M, pH 7.4) and used for the estimation of malonaldialdehyde (MDA) (Ohkawa et al., 1979) and endogenous glutathione (GSH) (Moron et al., 1979). A part of the homogenate was centrifuged at 2910*g* and the supernatant was used to estimate total protein (Bradford, 1976) and to assess superoxide dismutase (SOD) (Marklund and Marklund, 1974) and catalase (CAT) (Aebi, 1984) enzyme activities.

2.6. Determination of serum TNF-α level

Serum TNF-α level was measured using commercially available kit as per manufacturer's instructions.

2.7. Histopathological evaluation

Tissue sections of 5 μm thickness were stained with hematoxylin and eosin (H&E) and evaluated by a blinded pathologist under light microscope. Histological changes were graded and scored on the basis of tubular damage as described in previous study (Terada et al., 2013). Scoring is as follows: 0=none, 1= ≤ 10%, 2=11–25%, 3=26–45%, 4=46–75%.

2.8. Immunohistochemical analysis

IHC was done for tissue localization of caspase-3. Paraffin tissue sections were passed through xylene and graded series of ethanol to deparaffinize and rehydrate tissues, and then heated in a microwave to 95 °C for 10 min in citrate buffer (10 mM; pH 6.0) for antigen retrieval. Hydrogen peroxide (30% H_2O_2 in methanol) was added to quench any endogenous peroxidase activity in tissues. Later, sections were blocked with normal goat serum for 1 h at room temperature and were incubated with primary rabbit monoclonal antibody (mAb) against caspase-3 (1:1000) for 48 h. Sections were then incubated with horse radish peroxidase (HRP)-conjugated goat anti-rabbit IgG (1:3000) secondary antibody for 2 h. Colorimetric immune reaction was developed with 3,3′-diaminobenzidine (DAB). Sections were later (after 2 min) counterstained with hematoxylin