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The selective tyrosine kinase-inhibitor nilotinib alleviates experimentally induced cisplatin nephrotoxicity and heptotoxicity

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ABSTRACT

This work tested the action of nilotinib, selective inhibitor of tyrosine kinase on cisplatin (CP)-induced damage of kidney and liver in rats. Rats were assigned to 4 groups, control, nilotinib, CP, and CP plus nilotinib. Assessment of kidney and liver function, lipid peroxidation and antioxidant markers, anti-apoptotic protein Bcl2, nuclear factor- kappa B (NF-κB) immunoreactivity, and caspase 3 activity were done. CP-induced damage evidenced by histopathological changes, deterioration of renal and liver function, imbalance in oxidants/antioxidants markers, decreased Bcl2, increased caspase 3 activity, and NF-κB nuclear expression in both organs. Nilotinib treatment with CP restored kidney and liver oxidants/antioxidant levels also increased Bcl2 and decreased NF-κB immunoreactivity were evident with nilotinib treatment. In conclusions these results demonstrated a protective effect of nilotinib in experimentally induced CP kidney and liver damage that could be mediated through combating oxidative stress, reducing inflammation and anti-apoptosis in the two organs.

1. Introduction

The platinum-based compound Cisplatin (CP) is a frequently used anti-cancer for the treatment of many types of solid tumors. CP dose escalation is frequently required to improve its efficacy and overcome resistance imposed by tumor cells (Siddik, 2003). However, CP has serious dose-dependent adverse effects including nephrotoxicity, hepatotoxicity, germ cell toxicity, neurotoxicity and bone marrow suppression which limit its usefulness in the clinical settings (Santabarbara et al., 2016). Nephrotoxicity, a main adverse event associated with the drug is estimated to occur in nearly thirty percent of patients treated with CP (Pabla and Dong, 2008). Taking into account the multiple functions performed by the liver, injurious damage of this organ caused by high doses CP and long-term low doses is also considered an important adverse effect (Naqshbandi et al., 2012).

Oxidative stress, inflammation and apoptosis are thought to be important mechanisms mediating CP-induced organ toxicity of kidney and liver. CP induces reactive oxygen species (ROS) production which can be damaging to important cell components via their interaction with DNA, proteins and lipids. ROS triggers the redox sensitive transcription factor NF-κB that translocates to the nucleus to upregulate transcription of proinflammatory genes including many cytokines and chemokines. In addition, many studies documented activation of proapoptotic pathways in CP-induced nephrotoxicity and hepatotoxicity (Li et al., 2016; Omar et al., 2016; Sahu et al., 2013). The normal balance among pro- and anti-apoptotic pathways in the kidney and liver is shifted in favor of the proapoptotic pathways by CP. Regaining the normal balance through suppression of proapoptotic factors and/or increasing the antiapoptotic ones seems a way of protection against CP-toxicity in these organs (Malik et al., 2015; Omar et al., 2016). Therefore, to improve outcomes of CP treatment, it seems rational to study agents targeting the mechanisms behind toxicity.

Nilotinib is a selective tyrosine kinase inhibitor which belongs to the second generation of the group. It is 30-times more potent than the first generation of the class, imatinib (Breccia and Alimena, 2010). The drug is FDA-approved as a first line therapy for patient having Breakpoint Cluster Region-Abelson (BCR–ABL)-positive chronic myelogenous leukemia and as a second line for those who show resistance or intolerance to imatinib. It is a competitive inhibitor at the ATP-binding site of (BCR–ABL) and as a selective inhibitor of abelson kinase (c-Abl). It can also inhibit platelet-derived growth factor and stem cell factor receptors (c-kit) (Manley et al., 2010). Studies reported other favorable pharmacological effects of nilotinib including the ability to suppress oxidative damage and modulate inflammation and apoptosis pathways

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(Iyoda et al., 2011; Nader and Attia, 2016; Shaker, 2014). Based on these studies and the proposed mechanisms implicated in CP-induced kidney and liver damage in addition to the urgent need to find a drug to ameliorate or reverse CP-organ toxicity, the existing work was planned to scrutinize the potential ameliorative action of nilotinib in CP-induced nephrotoxicity and hepatotoxicity in rats.

2. Materials and methods

2.1. Drugs and chemicals

Nilotinib hydrochloride monohydrate with molecular formula: C 28H22 F 3 N7 O·HCl·H2O. The molecular mass is 583.99 (as monohydrate). It appears as a white to slightly yellowish or slightly greenish yellowish powder. The solubility of nilotinib hydrochloride monohydrate in aqueous solutions at 25 °C strongly decreases with increasing pH, and it is practically insoluble in buffer solutions of pH 4.5 and higher pH values. Nilotinib is sparingly soluble in ethanol and methanol. Cisplatin was acquired from Bristol-Myers- Squibb Co, (Princeton, NJ, USA) and dissolved in normal saline while Nilotinib was taken from Novartis Pharma (AG, Basel, Switzerland) and was dissolved in 0.5% CMC. Other chemical agents were of highly analytical grade.

2.2. Experimental animals

The Animal Care and Use Committee of the National Research Centre (Egypt, April 2009) approved the experimental protocol, and all procedures performed in this study were in agreement with ethical guidelines of the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 2011). Twenty eight male Wistar albino rats aged 8 weeks and weighed 180–220 g were maintained in cages under standard condition of temperature, humidity, hygiene and a12h-light/dark cycle at the animal house colony of the National Research Centre, Dokki, Egypt. The rats had free access to food and water.

2.3. Experimental design

After acclimatization for 1 week, rats were randomized to four groups containing six rats each except CP group containing 10 rats. Group I; control group, rats subjected to both drug vehicle (0.5% CMC) and CP vehicle (0.9% saline). Group II; nilotinib-group, rats treated with nilotinib (25 mg/kg/day, orally). Group III; CP- group, rats received single dose of CP (6 mg/kg, IP) and received drug vehicle orally. Group IV; Nilotinib-CP group, rats received both nilotinib and CP. Treatment with nilotinib began 4 days before and 6 days after CP single injection. The chosen nilotinib dose was chosen after preliminary data using lesser doses of nilotinib (5 & 15 mg/kg/day, orally). Our selected dose was chosen to produce the best results in ameliorating both renal and liver injury which were estimated by measuring serum creatinine (Cr) and blood urea nitrogen (BUN), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and mortality rate.

On the fourth day following CP administration, rats were located individually in metabolic cages for the measurement of urine volume over 24 h. The urine samples were used for Micro-total protein (MTP) and creatinine clearance (Cr-Cl) measurements.

After collection of urine, rats were subjected to the inhalational anesthesia with diethyl ether, then blood samples were withdrawn from retro orbital plexus and left to stand for half an hour. Blood samples centrifuged to separate serum that was used for measurement for different biochemical measurements at 1000*g* for 15 min at 4 °C. Then rats sacrificed by fast decapitation. The kidney and liver were dissected out and parts from them were mixed immediately in 10% phosphate buffered formalin for histopathological and immunohistochemical studies. Homogenization was carried out in ice-cold 0.1 M phosphate buffer (pH 7.4) for kidney tissues and 1.15% KCl solution for hepatic samples to yield 10% w/v tissue homogenates (Oncu et al., 2002).

2.3.1. Serum analysis

BUN, Cr, ALT, AST, gamma-glutamyl transferase (γGT), lactate dehydrogenase (LDH) and albumin were measured using commercial kits (BioMerieux, Marcy-l'Etoile, France). Also concentration of MTP and Cr-Cl in urine were assessed by applying commercial kits (Thermo Scientific, Rockford, USA).

2.3.2. Estimation of MDA in hepatic and renal tissue

The level of lipid peroxidation was estimated quantitatively by determination of MDA level in the supernatants of liver and kidney homogenates (Ohkawa et al., 1979). The level of MDA was expressed as nmol/g tissue.

2.3.3. Estimation of SOD and GSH in hepatic and renal tissue

Estimation of SOD was assayed utilizing the technique of (Marklund, 1985) the SOD activity was expressed as unit/g tissue, while the concentration of GSH was assayed colorimetrically (Ellman, 1959) in the supernatants of kidney and liver homogenate using Biodiagnostic kits (Giza, Egypt). The result of GSH was expressed as μM/g tissue.

2.3.4. Estimation of caspase3 in hepatic and renal tissue

The activity of both kidney and liver caspase3 was determined using a commercial colorimetric kit (Sigma Aldrich, USA) as per the standard methods provided. In brief, the assay depends on hydrolysis of the peptide substrate acetyl-Asp-Glu-Val-Asp *p*-nitroanilide (Ac-DEVD-pNA) by caspase 3, resulting in the release of the *p*-nitroaniline (pNA) moiety. *p*-Nitroaniline has a high absorbance at 450 nm. Optical density (OD) (the concentration of the pNA released from the substrate) is calculated from the calibration curve prepared with defined pNA solutions according to the manufacturer's instructions.

2.3.5. Assessment of histopathological findings

Tissue slices from kidney and liver were cut by using Tissue-Tek (ACCU-CUT MICROTOME) slices were fastening in 10% neutral buffered formalin, embedded in paraffin, and sectioned (4–5 μm). Hematoxylin and eosin (H & E) stain was used for slices staining, and then examined in an arbitrary order by using light microscope (Olympus).

2.3.6. Immunohistochemical staining of NF-κB and Bcl2 in renal and hepatic tissues

Immunostaining was done according to Avidin-Biotin complex (ABC) technique using (ImmunoCruz™ rabbit ABC Staining System, Santa Cruz Biotechnology, Catalog Number sc-2018) according to the producer's instructions (Scartozzi et al., 2007). The stained slices were examined in a random order.

2.4. Statistical analysis

Data are represented as mean ± SEM. Data were done by one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons test. P value of < 0.05 were considered statistically significant. All calculations were done by applying Instat-2 computer program (GraphPad Software Inc. V2.04, San Diego, CA, USA).

3. Results

After statistical analysis, results showed that nilotinib-treated group was not statistically significant from normal group in all measured parameters (data not shown). Therefore, this group was omitted from tables and figures to facilitate data presentation.