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Pharmacological inhibition of NADPH oxidase protects against cisplatin induced nephrotoxicity in mice by two step mechanism

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ABSTRACT

Background: Cisplatin induced nephrotoxicity is primarily caused by ROS (Reactive Oxygen Species) induced proximal tubular cell death. NADPH oxidase is major source of ROS production by cisplatin. Here, we reported that pharmacological inhibition of NADPH oxidase by acetovanillone (obtained from medicinal herb *Picrorhiza kurroa*) led to reduced cisplatin nephrotoxicity in mice.
Methods: In this study we used various molecular biology and biochemistry methods a clinically relevant model of nephropathy, induced by an important chemotherapeutic drug cisplatin.
Results: Cisplatin-induced nephrotoxicity was evident by histological damage from loss of the tubular structure. The damage was also marked by the increase in blood urea nitrogen, creatinine, protein nitration as well as cell death markers such as caspase 3/7 activity and DNA fragmentation. Tubular cell death by cisplatin led to pro-inflammatory response by production of TNFα and IL1β followed by leukocyte/neutrophil infiltration which resulted in new wave of ROS involving more NADPH oxidases. Cisplatin-induced markers of kidney damage such as oxidative stress, cell death, inflammatory cytokine production and nephrotoxicity were attenuated by acetovanillone. In addition to that, acetovanillone enhanced cancer cell killing efficacy of cisplatin.
Conclusion: Thus, pharmacological inhibition of NADPH oxidase can be protective for cisplatin-induced nephrotoxicity in mice.

1. Introduction

Cisplatin is commonly used anti-cancer drug in urinary bladder, ovarian and testicular cancers. It is also used to treat several other cancers including lung cancer. As an alkylating agent, cisplatin cause cell death to cancer cells after crosslinking to DNA. However, its chemotherapeutic efficacy is hampered by severe side effects especially nephrotoxicity (dos Santos et al., 2012; Joaquim et al., 2010). Kidneys are particularly affected by cisplatin due to (a) high amount of cisplatin accumulation in the kidneys and (b) the specific renal transport systems for cisplatin. Production of reactive oxygen species (ROS) by cisplatin in kidney is crucial to the progression of nephrotoxicity (Arany and Safirstein, 2003; dos Santos et al., 2012; Sanchez-Gonzalez et al., 2011). The major sources of ROS production in cisplatin nephrotoxicity are NADPH (nicotinamide adenine dinucleotide phosphate) oxidases (also known as NOX) and mitochondrial ROS (Chirino and Pedraza-Chaverri, 2009; Mukhopadhyay et al., 2012).

All NOX family members transport electron to reduce oxygen to superoxide. Superoxide is a primary ROS molecule. Superoxide may convert to more toxic ROS, such as hydrogen peroxide or peroxynitrite (Pacher et al., 2007). One of the inhibitors for NADPH oxidase is acetovanillone (also known as apocynin), which is a methoxy-substituted catechol derived and obtained from the root extract of the medicinal herb *Picrorhiza kurroa* (Stolk et al., 1994). Acetovanillone has been shown to confer protection in animal models of liver ischemia (Liu et al., 2008), arthritis (t Hart et al., 1991) and endotoxin-induced lung injury (Wang et al., 1994).

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Numerous scientific articles demonstrate the protective effect of common antioxidants, plant products and inhibitor of signaling pathways in cisplatin induced nephrotoxicity(Ali and Al Moundhri, 2006). Common antioxidant N-acetylcysteine, Vitamin E, Carvedilol protects against cisplatin induced renal damage (Abdelrahman et al., 2010; Appenroth et al., 1997; Carvalho Rodrigues et al., 2012; Carvalho Rodrigues et al., 2013; Nematbakhsh and Nasri, 2013; Riga et al., 2013). Broccoli derived sulforaphane, fruit derived fisetin, asian spice derived curcumin, capsaicin and arabic gum prevents cisplatin induced nephropathy (Al-Majed et al., 2003; Gaona-Gaona et al., 2011; Guerrero-Beltran et al., 2010, 2012; Jung et al., 2014; Kursunluoglu et al., 2014; Sahu et al., 2014; Trujillo et al., 2013). Transcriptional regulator NRF2 and its target heme oxygenase-1 have critical role in cisplatin mediated renal damage (Aleksunes et al., 2010; Sahin et al., 2010a, 2010b; Shiraishi et al., 2000). Another transcriptional regulator p53 also plays role in the same (Jiang and Dong, 2008; Kang et al., 2011). Mitochondria targeted antioxidants and its downstream PARP pathway inhibitors demonstrate protective effect against cisplatin toxicity in mice (Mukhopadhyay et al., 2011, 2012; Pan et al., 2015). Recently, we have demonstrated that metalloporphyrins protects against cisplatin induced renal injury in mice by reducing peroxynitrite formation (Pan et al., 2014).

In this study we aimed to understand the contribution of NADPH oxidases by pharmacological inhibition in a widely used mouse model of cisplatin nephrptoxocity. Here, we demonstrated that acetovanillone protected kidney from cisplatin induced renal damage and cell death. Our results indicated important role of NADPH oxidase in cisplatin induced apoptosis and cell death. We also demonstrated that acetovanillone did not promote cancer cell proliferation but instead it enhanced cancer cell killing efficacy of cisplatin.

2. Materials and methods

2.1. Ethics statement of the study

This study was done in strict accordance with recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All protocols were approved by the Committee on the Ethics of Animal Experiments of First Affiliated Hospital, College of Medicine, Zhejiang University (Permit Number: 09-028) at the Chinese Academy of Sciences. All efforts were made to minimize suffering.

2.2. Animal Experiments

Male mice(C57BL/6) of ~8 weeks age with weights of 18–22 g were used in all experiments. Each experimental group was composed of six mice. Animals were kept under constant temperature (25 °C) and humidity with a 12 h light/dark cycle and had access to food and water *ad libitum* throughout the study. Mice were sacrificed at 3 days (72 h) after a single injection of cisplatin (cis-diammine platinum (II) dichloride, Sigma) at dose 20 mg/kg i.p. in DMSO/saline vehicle. Acetovanillone (also known as apocynin) was purchased from Sigma Chemical. Drug was dissolved in saline and administered at 10 mg/kg (or as described in text), i.p, daily, starting 2 h before the cisplatin administration. Acetovanillone was administered alone (without cisplatin treatment) as a separate group. The experiments were repeated two more times to validate the data.

2.3. Measurements of serum BUN and creatinine

On the day of the sacrifice, blood was collected by cardiac puncture and serum levels of blood urea nitrogen (BUN) and creatinine were measured as described earlier (Pan et al., 2014).

2.4. Histological evaluation of kidney damage

Kidneys were fixed with 10% formalin for 24 h in an orbital shaker. Kidneys were sectioned and stained with periodic acid–Schiff (PAS) reagents for histological examination as described earlier (Pan et al., 2014). Tubular damage in PAS-stained sections was examined under the microscope and scored based on the

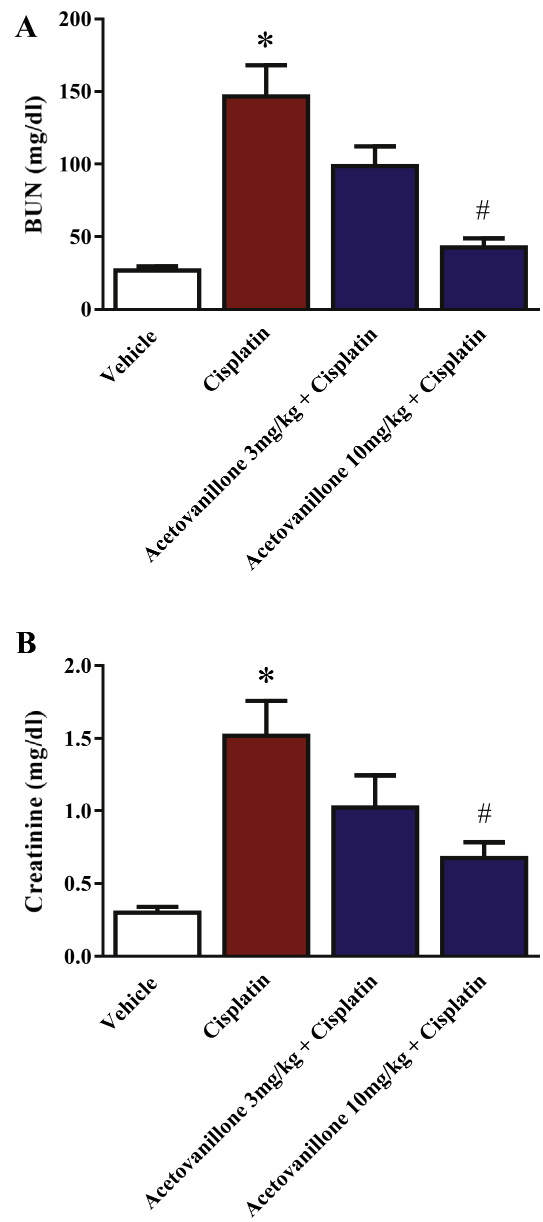

Fig. 1. Effect of acetovanillone on cisplatin-induced renal dysfunction in mice. Cisplatin caused significant renal dysfunction as measured by the levels of BUN (Panel A) and creatinine at 72 h (Panel B). Cisplatin administration resulted in severe kidney injury which was attenuated by acetovanillone treatment. Results are mean ± S.E.M. n = 4–5/group.*p < 0.05 versus vehicle; and #p < 0.05 versus cisplatin.