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Honokiol, a Polyphenol Natural Compound, Attenuates Cisplatin-Induced Acute Cytotoxicity in Renal Epithelial Cells Through Cellular Oxidative Stress and Cytoskeleton Modulations

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Cisplatin is a potent anti-cancer drug that has been widely used in the treatment of various cancers; however, cisplatin administration results in severe nephrotoxicity and impedes its clinical applications. In this study, we showed that honokiol, a polyphenol constituent extracted from *Magnolia officinalis* exhibited a short-term protective effect against cisplatin-induced damages in renal epithelial cells *in vitro*. The protective effects of honokiol were resulted from the combination of (1) reduced cellular oxidative stress ranging from 53 to 32% reduction during a 24-h incubation, (2) the maintenance of cellular antioxidant capacity and (3) the stabilization of cytoskeletal structure of the kidney epithelial cells. By promoting the polymerization of actin (1.6-fold increase) and tubulin (1.8-fold increase) cytoskeleton, honokiol not only maintained epithelial cell morphology, but also stabilized cellular localizations of tight junction protein Occludin and adhesion junction protein E-Cadherin. With stabilized junction protein complexes and structural polymerized cytoskeleton network, honokiol preserved epithelial cell polarity and morphology and thus reduced cisplatin-induced cell disruption and damages. Our data demonstrated for the first time that honokiol could counteract with cisplatin-induced damages in renal epithelial cells *in vitro*, future *in vivo* studies would further validate the potential clinical application of honokiol in cisplatin-based cancer treatments with reduced nephrotoxicity.

Keywords: honokiol, cisplatin, kidney, cytoskeleton, oxidative stress

INTRODUCTION

The kidney is an important organ responsible for waste elimination, electrolyte balance and erythropoietin production (Brown et al., 2012). Clinical symptoms for renal dysfunction include proteinuria, hypokalemia, metabolic acidosis and hyperphosphatemia (Chakrabarti et al., 2012). Both acute kidney injury (AKI) and chronic kidney disease (CKD) are characterized with different levels of functional or structural abnormalities of renal tissues. Kidney consists of various tubule structure formed by a single layer of epithelial cells that their apical membranes face toward the lumen where urine/water, endogenous waste and hormones are deposited to or secreted into (Brown et al., 2012), and their lateral membranes of adjacent renal epithelial cells are connected by junction protein complexes (Giepmans and van Ijzendoorn, 2009). To maintain this spatial segregation and orientation, epithelial cells must establish their cellular polarity. The generation of cell polarity is mediated by cell–cell adhesion and cell attachment to the extracellular matrix (Yeaman et al., 1999; Martin-Belmonte et al., 2008). Furthermore, the synthesis of signaling adhesion protein complexes at the sites of cell junctions, the organization of cytoskeleton and the constitutive sorting and transportation of proteins and molecules toward different membrane domains all contribute to the functional specialization of these asymmetry membrane regions and the maintenance of cell polarity (Giepmans and van Ijzendoorn, 2009). Alterations of kidney epithelial cell polarity have been reported in various pathological processes (Fish and Molitoris, 1994) and loss of cell polarity in kidney tubules upon the occurrence of AKI and CKD results in disruption of basic kidney tubular structure and defects of vital physiological functions (Thadhani et al., 1996).

Cisplatin has been used as an anti-cancer drug since 1970s (Dasari and Tchounwou, 2014). By forming inter- and intra-strand DNA adducts, cisplatin leads to apoptosis and necrosis of highly proliferating tumor cells (Chaney et al., 2005; Garcia Sar et al., 2012). Although cisplatin is one of the most effective platinum-containing therapeutic agents in treating various cancers (Desoize and Madoulet, 2002), its renal toxicity often impedes its clinical applications. The mechanism of cisplatin-induced cytotoxicity and organ failure is not only due to DNA damages of the cells, oxidative and nitrosative stresses induced by cisplatin have also been suggested (Santos et al., 2007; Dasari and Tchounwou, 2014). Cisplatin has been shown to provoke mitochondrial dysfunction that interfered the electron transport system and thus enhanced the generation of reactive oxygen species (ROS), nitrogen species (RNS) and led to mitochondrial dysfunction (Choi et al., 2015). Oxidative stress (OS) resulted from excessive amount of ROS and RNS generated by cisplatin is thought to participate in the pathogenesis of glomerular and tubular injuries in various renal diseases (Ozkok and Edelstein, 2014; Sureshbabu et al., 2015).

Defense mechanisms that protect cells from ROS-induced cell damage are crucial in both human and animal health. Besides traditional antioxidants (e.g., vitamin C or E), a number of exogenous antioxidants have also been used to against aging and neurodegeneration-related diseases (Youdim and Joseph, 2001; Agarwal et al., 2014). Among those of non-enzymatic antioxidants, honokiol (HNK), a small-molecule polyphenol constituent extracted from the bark of *Magnolia officinalis* exhibits several bioactivities including anti-allergy (Munroe et al., 2010), anti-anxiety (Guillermo et al., 2012; Sulakhiya et al., 2015), anti-cancer (Bai et al., 2003; Dasari and Tchounwou, 2014; Cheng et al., 2016), anti-depression (Jangra et al., 2015), and neuroprotection (Matsuda et al., 2001; Shen et al., 2010). Recent *in vitro* and *in vivo* studies showed that HNK exhibited anti-inflammation and anti-oxidant properties (Kim and Cho, 2008; Chao et al., 2010; Munroe et al., 2010; Li et al., 2011). Moreover, HNK is proved to be a multifunctional anti-oxidative molecule via its ability to reduce ROS production (Shen et al., 2010). On the basis of the aforementioned evidences, HNK is a promising compound to be exploited to attenuate cisplatin-induced renal toxicity and to improve clinical safety of cisplatin for patients who undergo cancer treatments. In this study, we aim to evaluate *in vitro*, the short-term (within 24 h treatment) effects of HNK on cisplatin-induced damages using renal epithelial cells as a testing model system.

MATERIALS AND METHODS

Chemicals, Reagents, Antibodies

Chemicals and reagents were obtained from Sigma-Aldrich (St. Louis, MO, United States) unless otherwise stated. *Cis*-Diammineplatinum(II) dichloride (Cisplatin, Cat. #479306, purity $\geq$ 99.9%) was purchased from Sigma. 2-(4-hydroxy-3-prop-2-enyl-phenyl)-4-prop-2-enyl-phenol (Honokiol, Cat. #SLK S2310, purity: 99.81%) was obtained from Selleckchem (Houston, TX, United States). Mouse monoclonal anti-E-Cadherin antibody (for immunofluorescence staining) was obtained from Cell Signaling Technology Inc. (MA, United States), rat monoclonal anti-E-Cadherin antibody (for Western-Blotting) was obtained from Sigma. Rabbit polyclonal anti-occludin antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, United States), mouse monoclonal anti-8-OHdG antibody and anti-GAPDH antibody was acquired from Abcam (Cambridge, United Kingdom) and Ambion (Invitrogen/Life Technologies, Carlsbad, CA, United States), respectively. For cytoskeleton detection and quantification, Alexa 568-Phalloidin and Alexa 488-anti-α tubulin antibodies were used (Invitrogen/Life Technologies). All secondary antibodies were purchased from Jackson ImmunoResearch (Jackson ImmunoResearch Laboratories Inc., West Grove, PA, United States).

Cell Culture

Stable cell line of Madin Darby Canine Kidney epithelial cell (MDCK) was purchased from American Type Culture Collection (ATCC, PTA-6500, Manassas, VA, United States) used throughout the study. Cells were cultured in Dulbecco's Modification of Eagle's Medium (DMEM, Gibco, NY, United States) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin-amphotericin B (Gibco) at 37.5°C in humidified atmosphere with 5% CO_2 for at least five