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NF-κB transcriptional inhibition ameliorates cisplatin-induced acute kidney injury (AKI)

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HIGHLIGHTS

- NF-κB transcriptional inhibition ameliorates kidney function and tubular necrosis.
- NF-κB transcriptional inhibition inhibits pro-inflammatory mediators.
- NF-κB transcriptional inhibition inhibits RIPK1 and 3.

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ABSTRACT

The nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) cell signaling pathway is important in inflammation and cell survival. Inflammation and cell death in the kidney are features of cisplatin-induced AKI. While it is known that cisplatin induces NF-κB signaling in the kidney, the NF-κB responsive genes and the effect of direct NF-κB transcriptional inhibition in cisplatin-induced AKI is not known. Mice injected with cisplatin, 25 mg/kg, developed AKI, acute tubular necrosis (ATN) and apoptosis on day 3. Mice were treated with JSH-23 (20 or 40 mg/kg) which directly affects NF-κB transcriptional activity. Kidney function, tubular injury (ATN, serum neutrophil gelatinase-associated lipocalin [NGAL], but not apoptosis) and myeloperoxidase (MPO) activity were significantly improved by JSH-23 (40 mg/kg). Sixty one NF-κB responsive genes were increased by cisplatin of which 21 genes were decreased by JSH-23. Genes that were decreased by JSH-23 that are known to play a role in cisplatin-induced AKI were IL-10, IFN-γ, chemokine [C-C motif] ligand 2 (CCL2) and caspase-1. Another gene, caspase recruitment domain family, member 11 (CARD11), not previously known to play a role in AKI, was increased more than 20-fold and completely inhibited by JSH-23. CXCL1 and TNF-α, known mediators of cisplatin-induced AKI, were decreased by JSH-23. RIPK1 and 3, receptor-interacting serine/threonine-protein kinases, that play an important role in necroptosis, were decreased by JSH-23. In mouse proximal tubule cells in culture, JSH-23 resulted in an increase in apoptosis suggesting that the mechanism of protection against AKI by JSH-23 is not due to a direct effect on proximal tubules. In conclusion, NF-κB transcriptional inhibition in cisplatin-induced AKI ameliorates kidney function and ATN without a significant effect on apoptosis and is associated with a decrease pro-inflammatory mediators and CARD11.

1. Introduction

Cisplatin is a widely used platinum-based chemotherapeutic agent with a dose-limiting renal toxicity (Ozkok and Edelstein, 2014). In patients, approaches that are used to prevent cisplatin-induced AKI are the use of a lower dose of cisplatin, administration of normal saline with resultant diuresis, use of carboplatin, a less toxic analog of cisplatin and treatment of hypomagnesemia (Ozkok and Edelstein, 2014). A better understanding of the mechanism of cisplatin-induced AKI, may result in the development of drugs that specifically prevent or treat cisplatin-induced AKI. Cisplatin-induced AKI has a multi-factorial mechanism including increased renal inflammation and apoptosis and necrosis of proximal tubular epithelial cells. The NF-κB pathway is an important family of transcription factors that

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control the expression of pro-inflammatory cytokines, chemokines and cell adhesion molecules and also mediates apoptosis, cell proliferation, differentiation and survival depending on the cell type and nature of the stimulus (Perkins, 2007; Hayden and Ghosh, 2008; Basak and Hoffmann, 2008; Barnes and Karin, 1997). As interstitial inflammation in the kidney involving neutrophils, T cells and cell death are features of cisplatin nephrotoxicity and the NF-κB pathway plays an important role in inflammation and cell death, the NF-κB pathway was studied in cisplatin-induced AKI.

While multiple studies have demonstrated cisplatin-induced activation of NF-κB in tubular cells both in vitro (Benedetti et al., 2013; Kim et al., 2012; Al-Lamki et al., 2012; Nozaki et al., 2011) and in vivo (Luo et al., 2008; Lee et al., 2006; Li et al., 2005; Bhat et al., 2002; Nozaki et al., 2012), the effect of direct inhibition of NF-κB in cisplatin-induced AKI is not known. As NF-κB directly mediates many pro-inflammatory and cell death pathways, that are also activated by cisplatin and NF-κB transcriptional inhibition represents a potential therapy for the prevention of cisplatin-induced AKI, the first aim of the study was to determine the effect of direct inhibition of NF-κB transcriptional activity on kidney function, kidney inflammation, tubular apoptosis and necrosis following the administration of cisplatin.

The activated form of NF-κB is a heterodimer, which consists of two proteins, a p65 subunit (also called Rel A) and a p50 subunit. NF-κB is a transcription factor that regulates the expression of many genes involved in immune and inflammatory processes and cell survival (Barnes and Karin, 1997; Hoesel and Schmid, 2013). Detailed information on all the NF-κB-responsive genes that are activated in kidney diseases, including AKI, is not known. Thus the second aim of the study was to determine which NF-κB-responsive genes were increased in the kidney in cisplatin-induced AKI and to determine the effect of NF-κB transcriptional inhibition on the NF-κB-responsive genes.

The pathological abnormalities in the kidney in cisplatin-induced AKI are tubular cell apoptosis and necrosis (Faubel et al., 2004). The relative contributions of direct tubular cell apoptosis and necrosis to the functional abnormalities in cisplatin-induced AKI is unknown. The third aim of the study was to determine whether NF-κB transcriptional inhibition had a direct effect to decrease tubular cell death in mouse proximal tubular cells in culture.

2. Materials and methods

2.1. In vivo model of cisplatin-induced AKI

For the animal studies, 8–10 week-old male C57BL/6 mice weighing 20–25 grams were used. Mice were chosen as they provide an excellent model that recapitulates the functional and histological features of cisplatin-induced AKI in humans. We have described this model of cisplatin-induced AKI in detail elsewhere (Faubel et al., 2004; Lu et al., 2007). Briefly, after 25 mg/kg cisplatin injection, BUN and serum creatinine are normal on day 1 and slightly increased on day 2. On day 3 after cisplatin injection, renal dysfunction, renal tubular cell apoptosis and acute tubular necrosis scores are severe. All experiments were conducted with adherence to the NIH Guide for the Care and Use of Laboratory Animals. The animal protocol was approved by the Animal Care and Use Committee of the University of Colorado at Denver. Mice were fed by a standard diet and water was freely available. Mice were housed 5 per cage under a 12 h light and dark schedule for at least one week prior to cisplatin administration. Six hours before cisplatin administration, food and water were withheld. Cisplatin [cis-Diamminedichloro-platinum(II)] (Aldrich, Milwaukee, WI, USA) was freshly prepared on the day of administration in sterile normal saline at a concentration of 1 mg/mL Mice were given 25 mg/kg body weight of cisplatin or vehicle (saline) intraperitoneally (IP), after which the mice again had free access to food and water. Mice were sacrificed on day 3 after cisplatin injection.

2.2. Preparation and administration of JSH-23

JSH-23 (NF-κB Activation Inhibitor II, JSH-23, catalog no:481408, Calbiochem-EMD Biosciences, Inc, San Diego, CA, USA) is an aromatic diamine (4-Methyl-*N*1-(3-phenyl-propyl)-benzene-1,2-diamine). JSH-23 was freshly prepared just before the injection. One vial of JSH-23 (5 mg) was dissolved in 500 μL of sterile 100% DMSO. Mice were given a total dose of either 20 mg/kg of JSH-23 (10 mg/kg 8 h prior to cisplatin injection and 5 mg/kg on days 1 and 2 after cisplatin injection) or a total dose of 40 mg/kg body weight of JSH-23 (20 mg/kg 8 h prior to cisplatin injection and 20 mg/kg on day 1 after cisplatin injection) or vehicle (DMSO). In all experiments, JSH-23 was administered to mice by intraperitoneal (IP) injection.

2.3. Histological examination

Paraformaldehyde (4%)-fixed and paraffin-embedded kidneys were sectioned at 4 μm and stained with periodic acid-Schiff (PAS) by standard methods. All histological examinations were performed by the renal pathologist in a blinded fashion. Histological changes due to acute tubular necrosis (ATN score) were evaluated in the outer stripe of the outer medulla on PAS- stained tissue and were quantified by counting the percent of tubules that displayed cell necrosis, loss of brush border, cast formation and tubule dilatation as follows: 0 = none, 1 = <10%, 2 = 10–25%, 3 = 26–45%, 4 = 46–75% and 5 = >75%. At least 10 fields (×250) were reviewed for each slide.

Morphologic criteria were used to count apoptotic cells on PAS-stained tissue by the pathologist experienced in the evaluation of renal apoptosis. Morphologic characteristics included cellular rounding and shrinkage, nuclear chromatin compaction and formation of apoptotic bodies (Gobe et al., 2000). Apoptotic tubular cells were quantitatively assessed per ten high power fields (HPF) (x400) in the outer stripe of the outer medulla by the renal pathologist in a blinded fashion.

2.4. BUN and creatinine measurements

Serum urea nitrogen and creatinine levels were measured using a Beckman autoanalyzer (Beckman Instruments, Fullerton, CA).

2.5. ELISA

Serum NGAL levels were determined by an NGAL enzyme-linked immunosorbent assay (ELISA) kit (Mouse Lipocalin-2/NGAL Immunoassay, R&D Systems). ELISA was performed according to the manufacturer's instructions.

2.6. Myeloperoxidase Activity (MPO) assay

Kidney tissue was homogenized in 1 ml of cold hexdecyltrimethylammonium bromide buffer (50 mM KPO4 and 0.5% hexdecyltrimethylammonium bromide [pH 6.0]), sonicated on ice for 10 s, and centrifuged at 14,000 g for 30 min at 4 °C. Twenty microliters of supernatant was transferred into a 96-well plate, and 200 μl of 37 °C O-dianisidine hydrochloride solution (16.7 mg O-dianisidine, 100 ml: 90% water and 10% 50 mM KPO4 buffer + 0.0005% H2O2) was added immediately before the optical density was read at 450 nm and again 30 s later (Benchmark microplate reader; BioRad).