KSN 2017 Abstract

KSN-17-O003

Oxidative stress caused by activation of NADPH oxidase 4 promotes colisitn-induced acute kidney injury

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Objectives : Colistin (polymyxin E) is an important constituent of the polymyxin class of cationic polypeptide antibiotics. Colistin induced nephrotoxicity may occur, in part, as a result of intrarenal oxidative stress. Nicotinamide adenine dinucleotide 3-phosphate (NADPH) oxidases (Noxs) are important sources of reactive oxygen species (ROS). Among various type of Noxs, Nox4 is expressed predominantly in the kidney. We investigated the role of Nox4 and the benefit of inhibition of Nox4 on colistin induced nephrotoxicity in vivo and in vitro models.

Methods : HK-2 cells were incubated with colistin at a concentration of 50 ug/ml for 24 h. Reverse transcription polymerase chain reaction for Nox4 was performed. Western blotting for Nox4, mitogen-activated protein kinases (MAPKs) and apoptotic pathway were done. HK-2 cells were transfected with Nox4 siRNA and pretreated with GKT137831 (most specific Nox1/4 inhibitor). The effect of Nox4 inhibition in HK-2 cells was observed with MTT assay and caspase 3/7 activity. ROS production was detected using a DHE stain and amplex red assay. In-vivo study were performed with Sprague-Dawley rats with or without GKT137831 colistin induced nephropathy model. Biochemical markers, histological examinations, 8OHdG and TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) stain were performed.

Results : Expression of Nox4 in human proximal tubular epithelial (HK-2) cells significantly increased following colistin exposure. Silencing of the Nox4 gene reduced the production of ROS, down regulated pro-inflammatory markers (mitogen-activated protein kinase, MAPKs) implicated in colistin induced nephropathy and attenuated apoptotic pathways (Bax and caspase 3/7 activity). Finally knocking down of Nox4 increased cellular survival in colisitn exposed HK-2 cells. Pretreatment with GKT137831, most specific Nox1/4 inhibitor, replicated theses effects by downregulation of MAPKs. In colistin induced

nephropathy rat model, co-treatment of GKT137831 with colistin resulted in attenuated colistin induced nephropathy as evidenced by attenuated the impaired glomerular function, increased superoxide dismutase (SOD) activity, preserved structure, reduced expression of 8OHdG and TUNEL positive cells.

Conclusions : Collectively, these results identify Nox4 as a key source of ROS responsible for kidney injury in colistin induced nephrotoxicity and provide a unique opportunity for treatment of drug related toxic nephropathy.

Keywords : Nox4, colistin, acute kidney injury