Pharmacologic Inhibition of NADPH Oxidase Nox4 Provides Renoprotection in Contrast Induced Nephropathy

Se-Hee Yoon[1], Won-Min Hwang[1],Bo Young Jeong[2], Hoi Young Lee[2], Chang Gyo Park[2], Doo-ri Choi[3], Seung-Yun Han[3], Moon Hyang Park[4], Soo Yong Lee[1], Sung-Ro Yun[1]

[1]Department of Internal Medicine, College of Medicine, Konyang University, Daejeon, Korea; [2]Department of Pharmacology, College of Medicine, Konyang university, Daejeon, Korea; [3]Department of Anatomy, College of Medicine, Konyang University, Daejeon, Korea; [4]Department of Pathology, College of Medicine, Konyang University, Daejeon, Korea

Introduction

Contrast media (CM) induced nephropathy (CIN) is an acute deterioration of renal function following administration of CM in the absence of any other known reason. CIN remains a leading cause of iatrogenic acute kidney injury (AKI), despite adherence to protocols of risk assessment and prevention strategies. CIN may occur, in part, as a result of intrarenal oxidative stress. NADPH oxidases are important sources of reactive oxygen species (ROS). Among various type of NADPH oxidases, Nox4 is expressed predominantly in rodent kidney.

Objectives

The aim of the present study was to assess the effect of Nox4 inhibition on the prevention of contrast induced nephropathy (CIN).

Methods

Using HK-2 cells, Nox4 mRNA and protein were determined after exposure to iohexol with/without pretreatment of the most specific Nox1/4 inhibitor, GKT137831. Caspase3/7 activity, DHE stain and amplex red activity were also measured. Proinflammatory and apoptotic markers (pNFkB/NFkB, pp38/p38, pJNK/JNK, pERK/ERK and pcleaved caspase/caspase) were measured for investigation of intracellular pathway associated with Nox4. In addition, the effect of Nox4 inhibition were evaluated in mice model of CIN.

Results

Figure 1. Nox4 expression after iohexol exposure was measured by quantitative real-time PCR and western blot. Nox4 expression was significantly increased following iohexol treatment within 30 minutes.

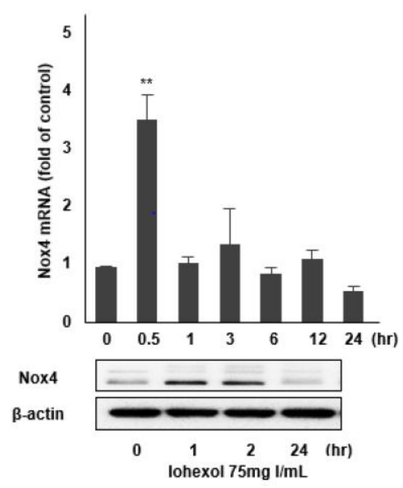


Figure 2. Nox4 is involved in contrast induced proximal tubular cell apoptosis. HK-2 cells were pre-treated with Nox1/4 inhibitor GKT137831. Treatment with GKT137831 at 20ug/mL abolished the apoptotic response induced by iohexol as indicated by caspase 3/7 activation.

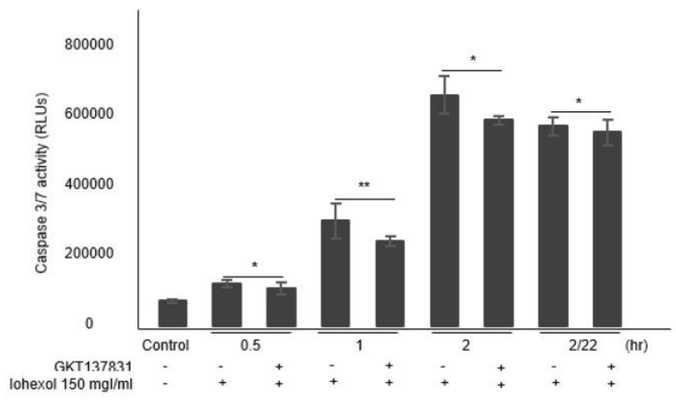


Figure 3. Silencing of the Nox4 gene improved the survival of HK-2 cells in response to iohexol as measured with ATPlite assay. Pretreatment of GKT137831 replicated theses effects also.

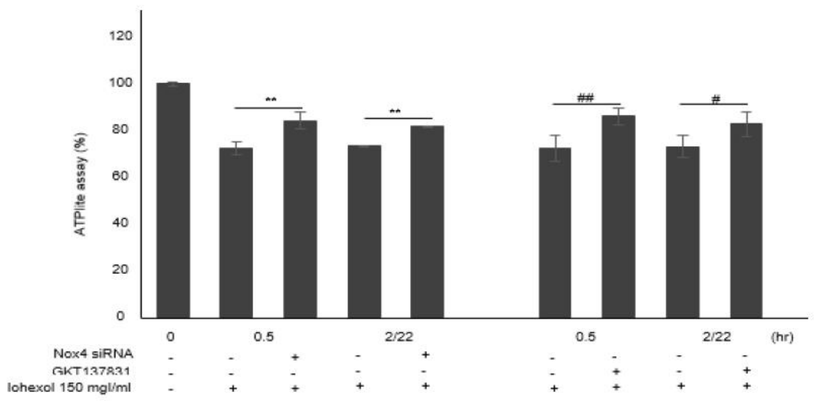
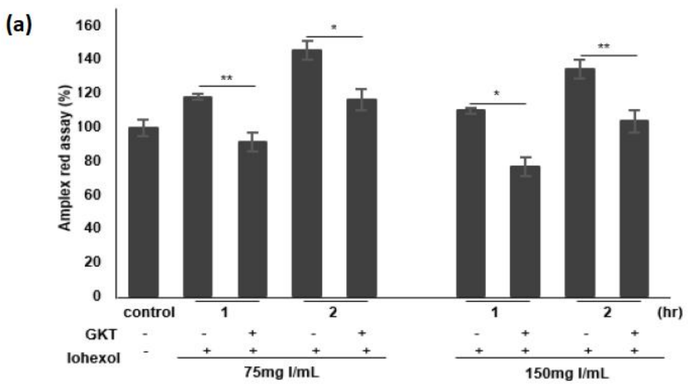
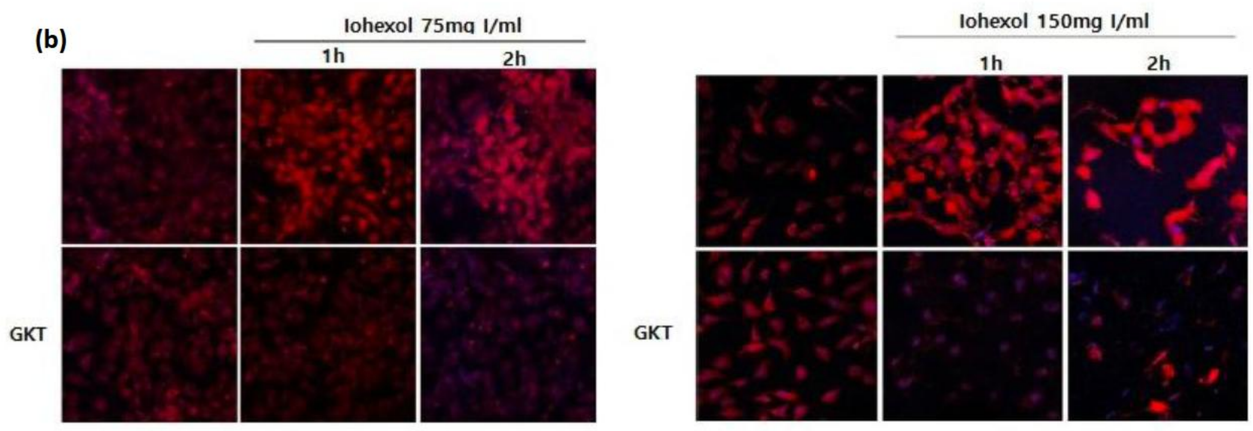


Figure 4. Iohexol induced apoptosis is dependent on ROS by Nox4



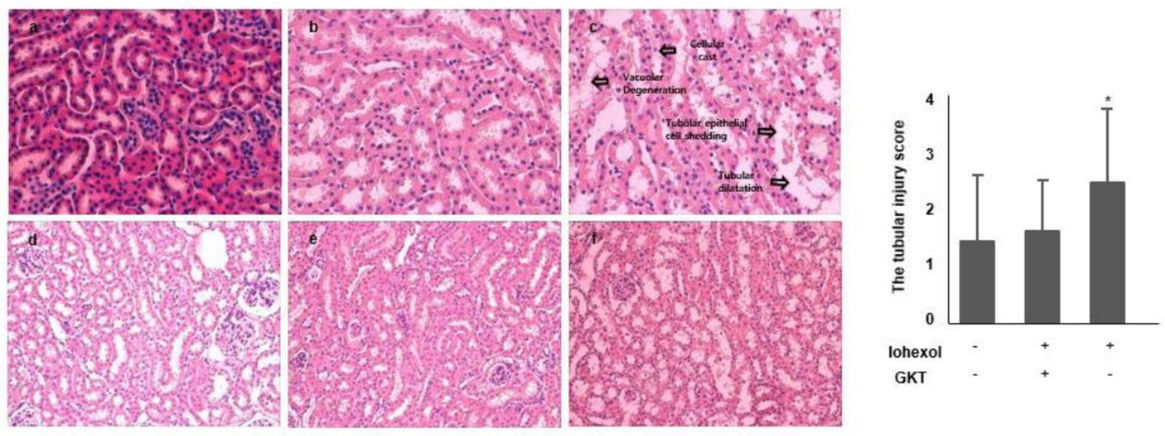


(A) The effect of iohexol on Nox4 expression were blocked by GKT137831 with amplex red assay. (B) Using Dihydroergotamine (DHE) fluorescence, we found that iohexol treatment enhanced intracellular ROS production in HK-2 cells, and this effect could be blocked by GKT137831.

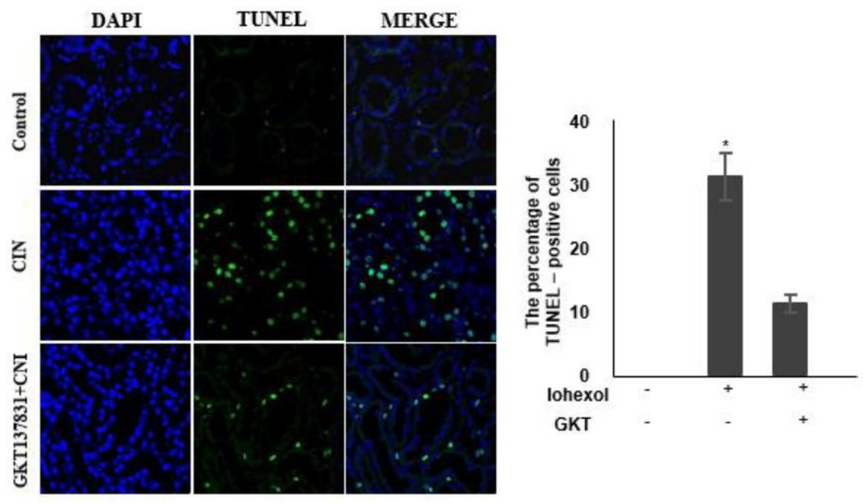
Figure 5. P38 MAPK mediates the redox-sensitive, iohexol induced proximal tubular cell apoptosis.

. (A) Iohexol (75 mg I/mL and 150 mg I/mL) treatment increase phosphorylation of p38 from 1h, and the level of p38 phosphorylation remained elevated up to 24h as compared with untreated cells. It was also observed that levels of p38 phosphorylation were elevated in iohexol treated cells at 23 h and 22 h after removal of the iohexol. (b) Inhibition of the Nox4 function with GKT137831 diminished contrast induced p38 phosphorylation (c) Knocking down Nox4 with specific siRNAs replicated this results.

Figure 6. Treatment with GKT 137831 *in Vivo* attenuated contrast induced tubular injury and apoptosis.



(A-F)The marked tubular injuries caused by iohexol are diminished by pretreatment with GKT137831. Representative photomicrographs of HE –stained kidney sections are presented Control mouse (A and D) mouse treated with iohexol (B and E) mouse treated with iohexol after GKT137831 treatment(C and F). Figures are representative of eight mice in each group. Magnifications: x 400 in A through C; x 200 in D through F.



Administration of GKI137831 inhibits renal tubular apoptosis caused by contrast medium. The induction of iohexol induced nephropathy increased the number of TUNEL-positive renal tubular cells, but the number is markedly decrease by preadministration of GKT137831.

Conclusions

Collectively, these results identify Nox4 as a key source of ROS responsible for kidney injury in contrast induced nephropathy and provide proof of principle for an innovative small molecule approach to prevent contrast induced nephropathy.

53 ERA
179--SP
Acute Kidney Injury. Experimental.
Se-Hee Yoon
DOI: 10.3252/pso.eu.53era.2016
ePosters supported by F. Hoffmann- La Roche Ltd.
53rd CONGRESS ERA-EDTA VIENNA AUSTRIA MAY 21st-24th, 2016
Held jointly with Österreichische Gesellschaft für Nephrologie
PosterSessionOnline.com