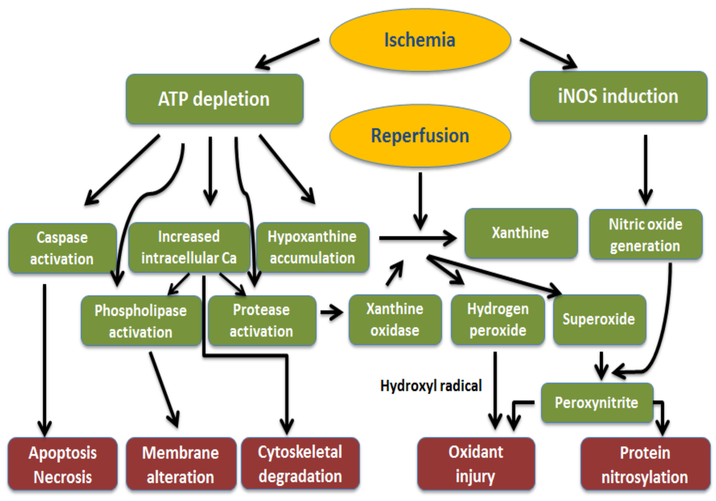
Ischemia reperfusion injury induces a pro-fibrotic phenotype in human proximal tubular epithelial cells

Rishab Kapoor, Ian Logan, John Kirby, Neil Sheerin
ICM, Newcastle University, Newcastle upon Tyne, United Kingdom

Introduction

Ischemia-reperfusion injury (IRI) is one of the major causes of acute kidney injury (AKI) and is associated with increased morbidity and mortality. During Ischemia the restriction in blood flow is more prominent in medulla than the outer cortex region of kidney. Reperfusion injury develops hours or days after the initial insult that is associated with upregulation of kidney pro-fibrotic protein markers. The initial insult followed by reperfusion leads to endothelial damage and dysfunction and vascular congestion that induces a hypoxic microenvironment and prevents clearance of toxic radicals. Thus proximal tubular region and medullary region are the most susceptible to kidney injury, inflammation and vascular damage.

Pathophysiology of IRI. *Alterations in kidney metabolism after ischemic acute kidney injury*



Aims

1. Establish a model of ROS-mediated and hypoxia-induced injury to proximal tubule epithelial cells focusing on expression of pro-fibrotic proteins.

2. To examine whether changes in epithelial cell phenotype and profibrotic markers are a direct consequence of IRI or whether the changes rely on the synthesis and/or activation of intermediates such as TGF-β.

Methods

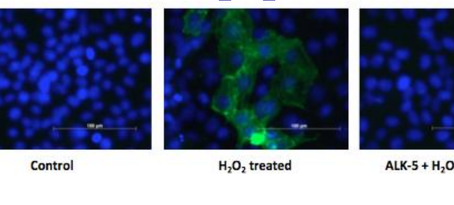
Immunofluorescence and western blot was performed post stimulating HKC8 and HK2 cells (human-derived renal proximal tubular cell line) with H_2O_2 and $CoCl_2$ treatment.

HKC8 cells transfected with luciferase gene were treated with H_2O_2 and $CoCl_2$ and luciferase assay was performed

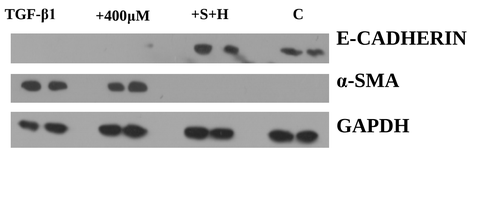
Western blot results

HKC8 cells

200 µM H_2O_2 treated- 72 hrs



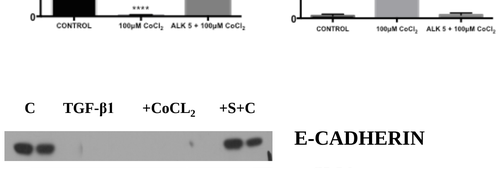
100 µM $CoCl_2$ treated - 96 hrs



HK-2 cells

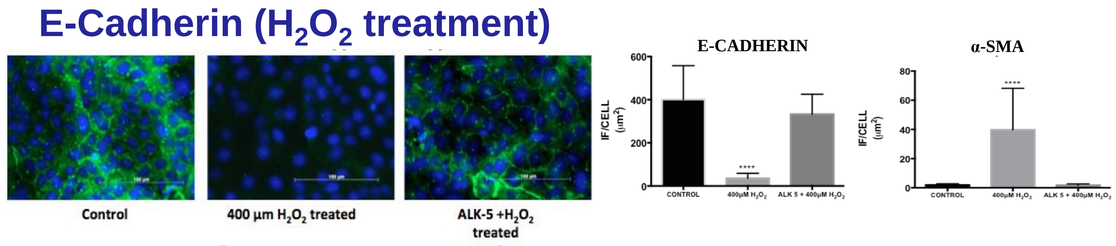
100 µM H_2O_2 treated- 72 hrs

100 µM $CoCl_2$ treated- 96 hrs



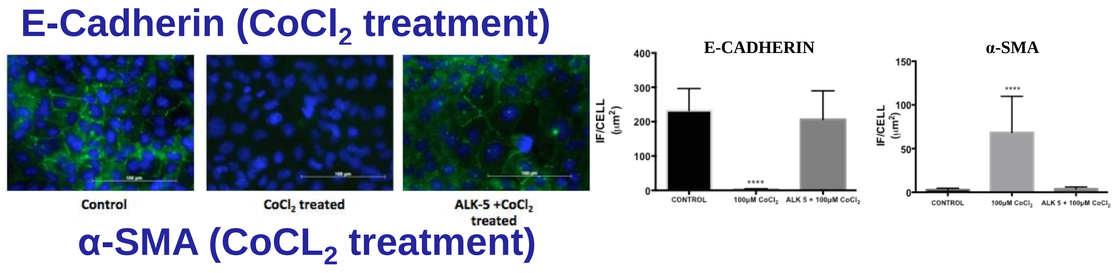
ALK-5 inhibitor studies in HKC8

E-Cadherin (H_2O_2 treatment)

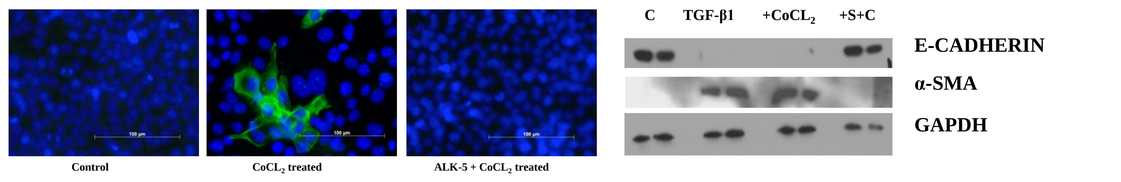


α-SMA (H_2O_2 treatment)

E-Cadherin ($CoCl_2$ treatment)



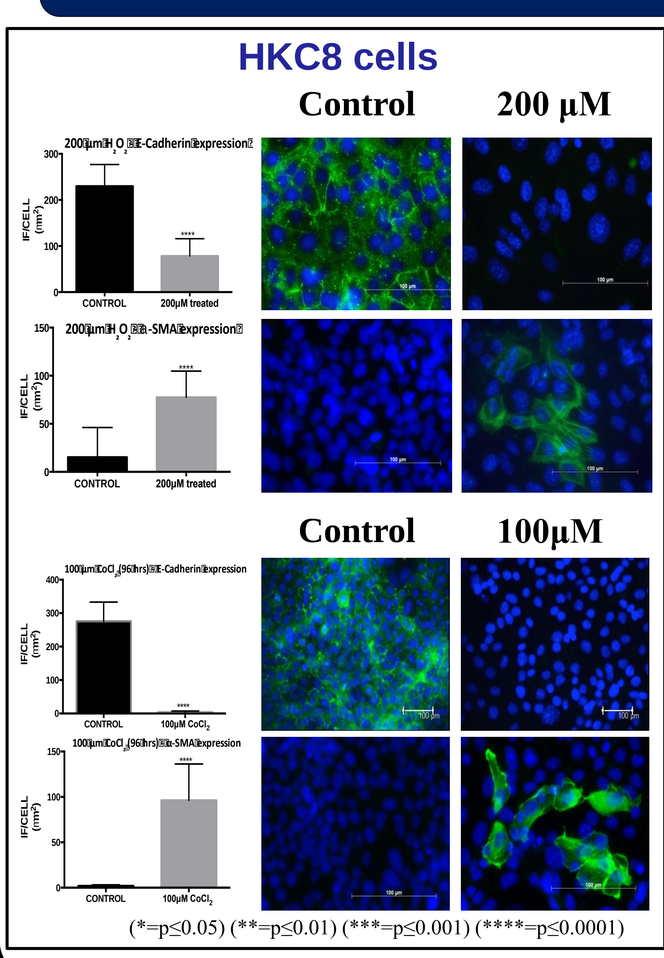
α-SMA ($CoCL_2$ treatment)



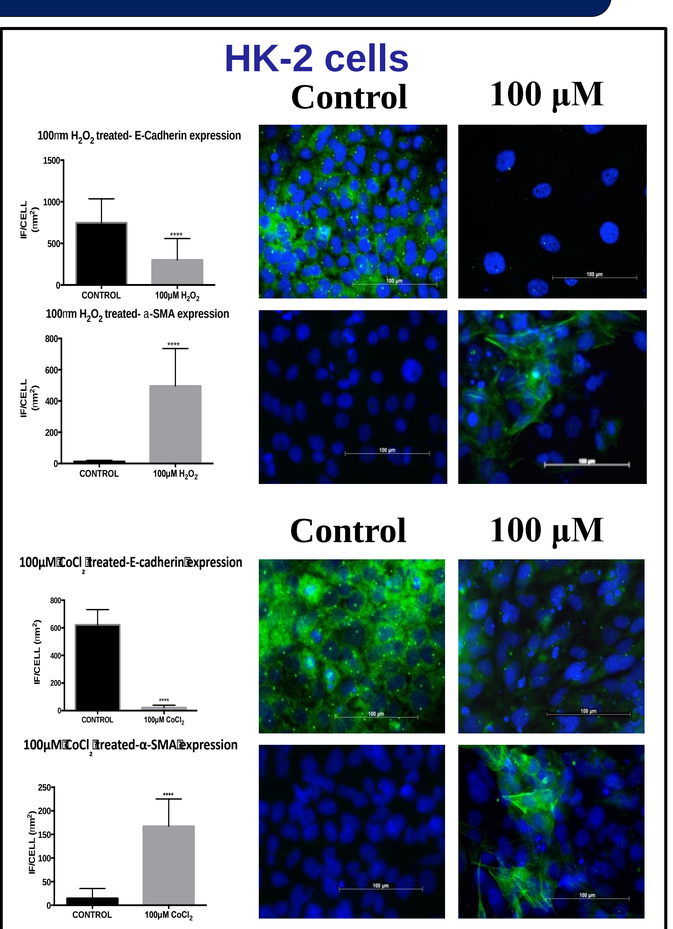
(*=p≤0.05) (**=p≤0.01) (***=p≤0.001) (****=p≤0.0001)

Immunofluorescence results

HKC8 cells



HK-2 cells

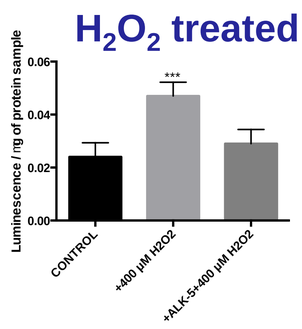


(*=p≤0.05) (**=p≤0.01) (***=p≤0.001) (****=p≤0.0001)

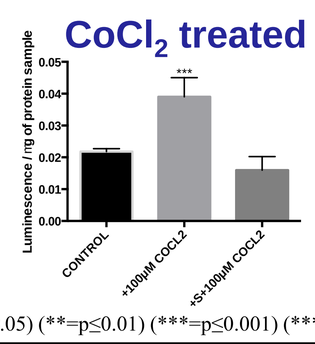
Luciferase assay

Results showed a significant ($p<0.001$) increase in luciferase activity (controlled by a SMAD promoter) post treatment with H_2O_2 and $CoCl_2$ as compared to cells preconditioned with media enriched with Alk-5 inhibitor.

H_2O_2 treated



$CoCl_2$ treated



(*=p≤0.05) (**=p≤0.01) (***=p≤0.001) (****=p≤0.0001)

Conclusion

1. Protein studies confirmed that HKC8 and HK2 show similar trend of extracellular matrix protein expression post stimulation with H_2O_2 and $CoCl_2$.

2. Alk-5 inhibitor (SB-505124) maintained the epithelial phenotype in H_2O_2 and $CoCl_2$ treated HKC8 cells suggesting the involvement of TGF-β in ischemia and reperfusion injury. This was confirmed using protein studies and luciferase assay.

53 ERA
178--MP
Acute Kidney Injury. Experimental.
Rishab Kapoor
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