MITOCHONDRIAL HOMEOSTASIS IS IMPEDED BY DEGRADATION AND AUTOPHAGY IN OXIDATIVE-STRESS INDUCED RENAL CELL INJURY

[1]David Small, [1]Nigel Bennett, [1,2]Jeff Coombes, [1,3]David Johnson and [1]Glenda Gobe

[1]Centre for Kidney Disease Research (CKDR), School of Medicine, Translational Research Institute and [2]School of Human Movement Studies, The University of Queensland, and [3]Dept of Nephrology, Princess Alexandra Hospital, Brisbane Australia

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

Background

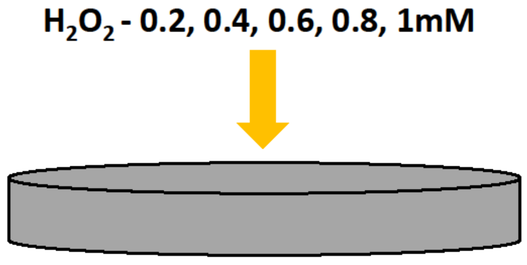
Oxidative stress may act in the pathogenesis of chronic kidney disease (CKD) by deregulating mitochondrial genes. Balancing degradation of defective mitochondria (autophagy) with renewal of healthy mitochondria (biogenesis) is vital for healthy cellular function. The adaptor protein p62 is implicated in degradation of damaged mitochondria. The nuclear transcription factors PPARγ and PPARα are responsive to oxidative stress and implicated in mitochondrial biogenesis. Our *aim* was to investigate oxidative stress-induced human kidney proximal tubular (PT) epithelial dysfunction, involving mitochondrial homeostasis, PPARγ, PPARα and p62 and expression and activation

Method

***In vitro* model of oxidative stress-induced kidney disease:**

- **HK-2 PT cells treated with hydrogen peroxide (H_2O_2; 0.2–1.0mM) for 2h and 18h (N=3)**
- **PPARγ agonist Troglitazone was used to determine functional significance of alterations in PPARγ expression and activation**

H_2O_2 - 0.2, 0.4, 0.6, 0.8, 1mM



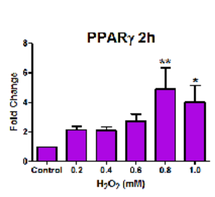
phospho-PPARγ, PPARγ, PPARα, p62, LC3-II (Westerns blot, immunofluorescence)

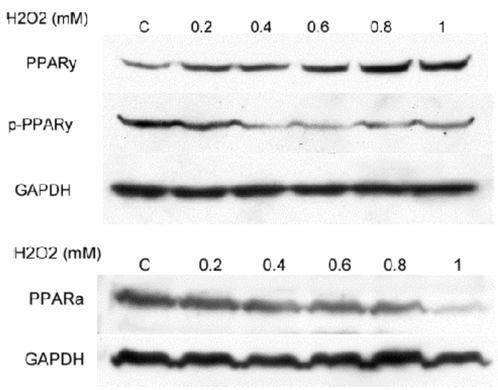
Morphological quantification of apoptosis & mitosis

ATP Assay, MitoTracker CMXRos (mitochondrial homeostasis)

1. Altered PPARγ and α Activation

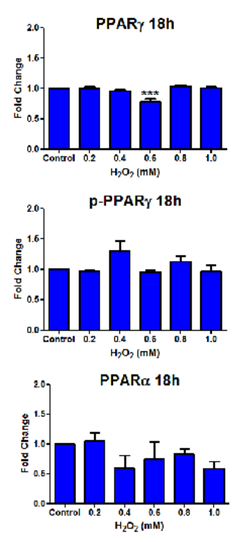
Short-term oxidative stress

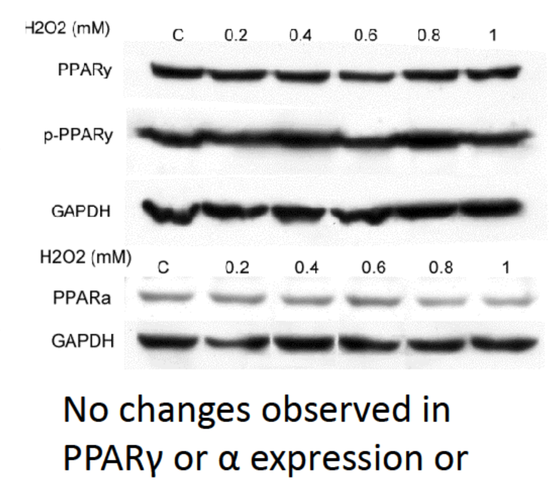




Initial (2h) upregulation of PPARγ but decreased activation (p-PPARγ) demonstrates impaired PPARγ regulation following oxidative stress.

Long-term oxidative stress

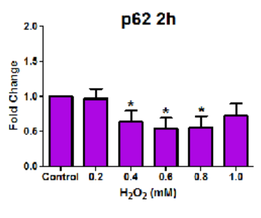


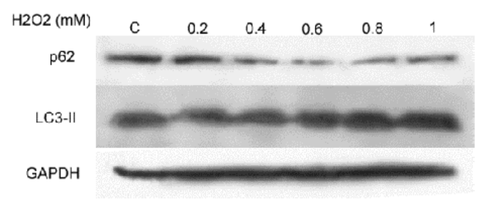


No changes observed in PPARγ or α expression or activation following long-term (18h) oxidative stress.

2. Altered p62 Expression

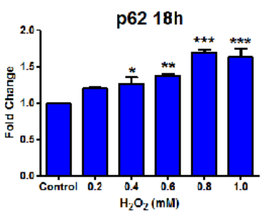
Short-term oxidative stress

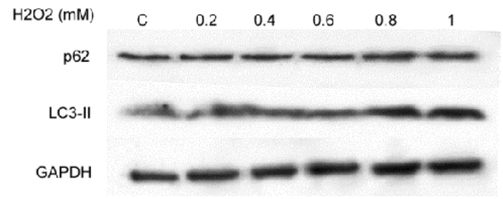




Initial decrease in p62 expression may indicate impaired mitochondrial autophagy following 2h oxidative stress.

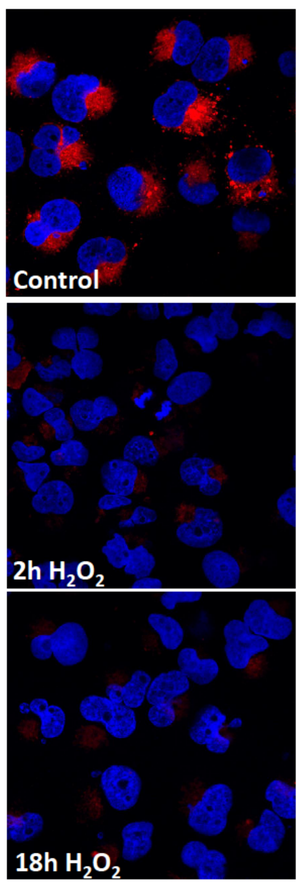
Long-term oxidative stress

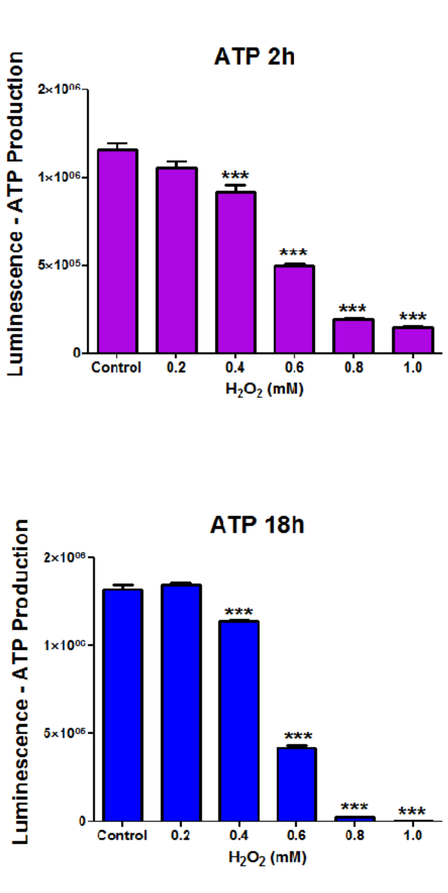




Increase in p62 and LC3-II expression indicates enhanced mitochondrial autophagy with longer-term 18h oxidative stress.

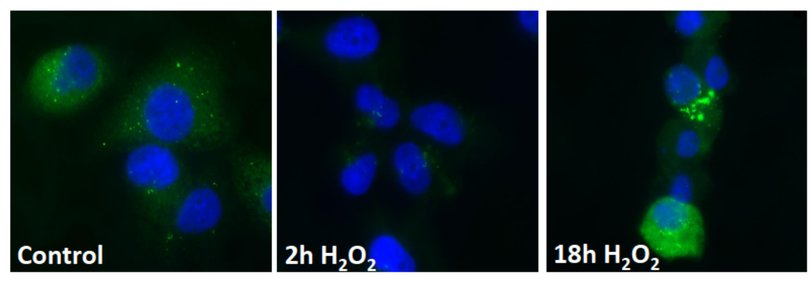
3. Altered Mitochondrial Homeostasis



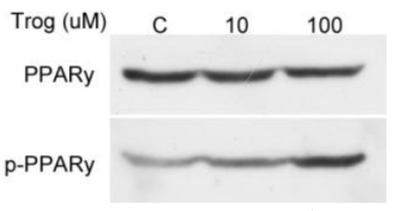


MitoTracker CMXRos
DAPI

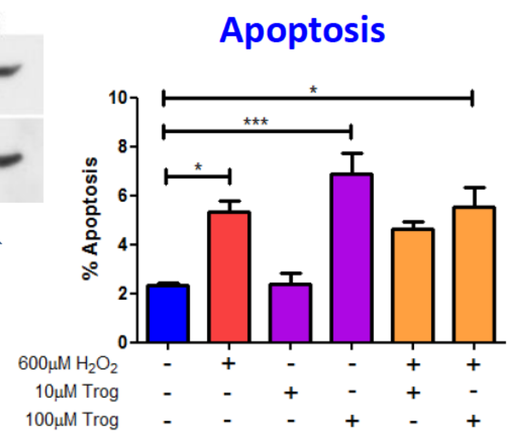
Decreased ATP production and MitoTracker uptake demonstrates mitochondrial dysfunction following short and long-term oxidative stress.



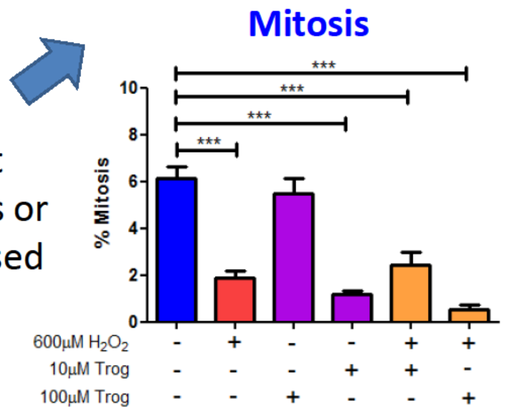
4. Pharmacologic PPARγ Activation (Troglitazone)



2h Troglitazone exposure caused a dose dependent increase in PPARγ activation.



2h pre-exposure to Troglitazone did not attenuate apoptosis or restore mitosis caused by oxidative stress.



Summary

Oxidative stress promotes mitochondrial destabilisation in human kidney PT epithelial cells with an early loss of p62 expression, and impaired PPARγ activation. Failure to remove damaged mitochondria via autophagy, or defective p62, may lead to a spiralling cycle of oxidative stress due to increased amounts of dysfunctional mitochondria that result in progressive deterioration of tubular function in CKD. Despite positive outcome in other tissues, activation of PPARγ may not be cytoprotective against oxidative stress in kidney PT epithelium.

50ERA-EDTA
364--SP
Experimental models of CKD
Glenda GOBE
DOI: 10.3252/pso.eu.50era.2013
Poster presented at: ERA-EDTA 50th Congress Istanbul, Turkey May 15-21, 2013
PosterSessionOnline.com