Cyclosporin A Acts Directly on Proximal Tubular Cells Leading to a Pathogenic Phenotype

S Jain[1], IA MacPhee[2], MK Phanish[1], ME Dockrell[1]
[1]South West Thames Institute for Renal Research, St Helier Hospital, London; [2]St George's University, London

INTRODUCTION

Cyclosporin A (CsA), is a potent immunomodulator used in solid organ transplantation and autoimmune conditions.

Long term use is associated with interstitial fibrosis and hence Chronic Kidney Disease.

This is thought to be due to its action on the vasculature causing local renal ischaemia although direct effects on renal proximal tubule cells (PTECS) have been reported.

This work demonstrates the phenotypic changes induced in PTECS after prolonged exposure to CsA *in vitro* and provides insight into the mechanism of these changes.

METHODS

Primary human PTECS were treated with CsA 10ug/ml for 1-144hrs.

Outcomes were compared with Vehicle conditions or with PTECS treated with TGFβ1 5ng/ml.

For treatments of 144hr, the medium was changed at 72hrs with continued exposure to CsA but not TGFβ1.

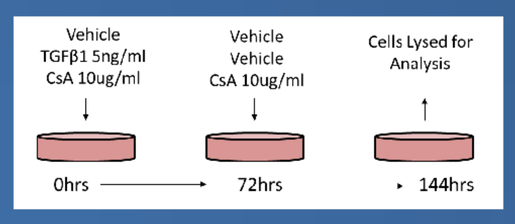
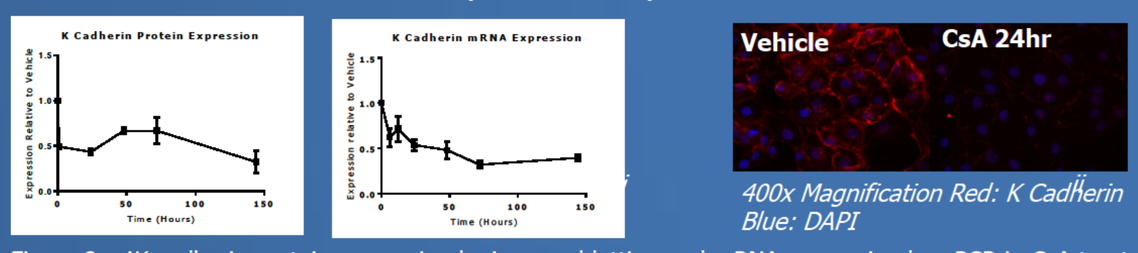


Figure 1: Cell culture medium is changed after 72 hours

RESULTS (1)

Expression of K cadherin, an important junctional protein in maintaining the integrity of the proximal tubule epithelium, was reduced in CsA treated cells as early as 1hr after exposure.

This was accompanied by decreased mRNA production; leading to a sustained decrease in protein expression.

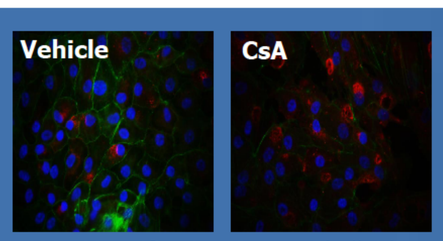


400x Magnification Red: K Cadherin Blue: DAPI

Figure 2: *i* K cadherin protein expression by immunoblotting and mRNA expression by qPCR in CsA treated PTECS relative to Vehicle conditions over time; *ii* Reduced cell membrane immunostaining of K cadherin after 24hrs CsA exposure

RESULTS (2)

After 144hrs, CsA treated cells became elongated and expressed the pro-fibrotic markers αSMA and Connexin 43.



400x Magnification Green: Phalloidin Red: αSMA

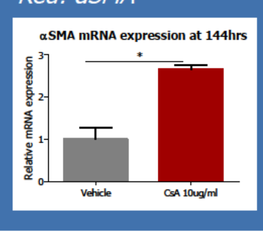
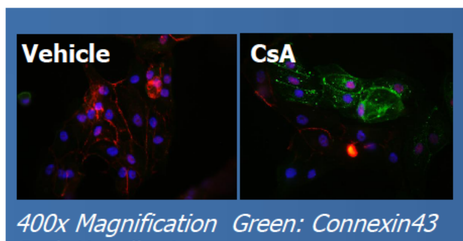


Figure 3: αSMA immunostaining and mRNA expression (graph) in CsA treated cells after 144hrs (*p<0.01)



400x Magnification Green: Connexin43 Red: K cadherin

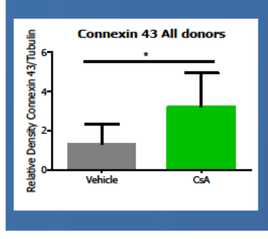


Figure 4:Connexin 43 immunostaining and protein expression (graph) in CsA treated cells after 144hrs (*p<0.01)

RESULTS (3)

As with treatment with the fibrokine TGFβ1, CsA induced MMP2 and 9, and inhibited MMP7 suggesting excess extracellular matrix production but unlike TGFβ1 treatment, CsA did not lead to increased production of Fibronectin, PAI1 and CTGF (Figure 5), suggesting independent processes.

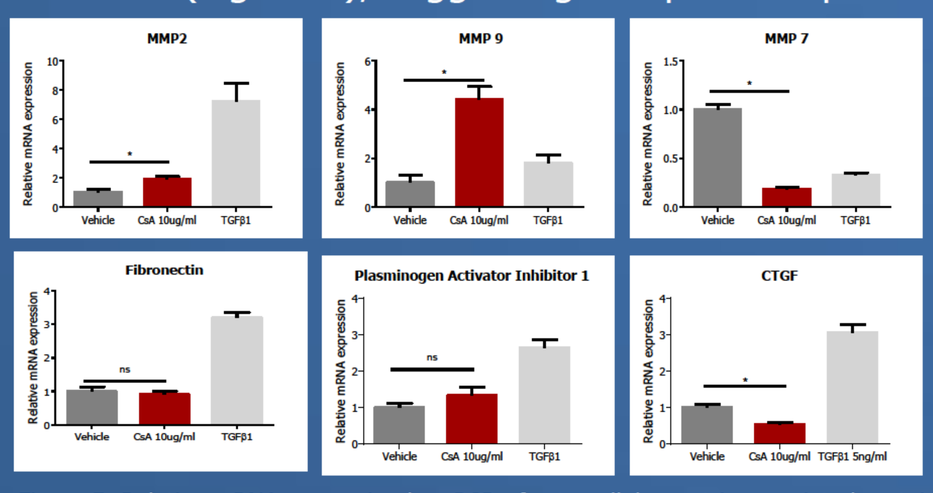


Figure 5: Relative mRNA expression by qPCR of extracellular matrix associated proteins and cytokines (*p<0.05)

This was confirmed by pre and co incubation with the Type 1 TGFβ1 receptor, SB431542 which prevented phenotypic changes in TGFβ1 treated PTECs but not CsA treated PTECs (Figure 6).

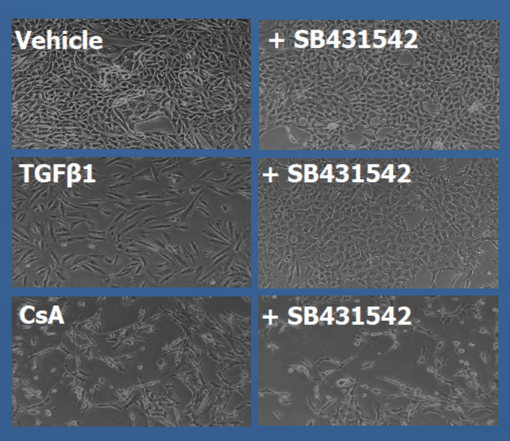
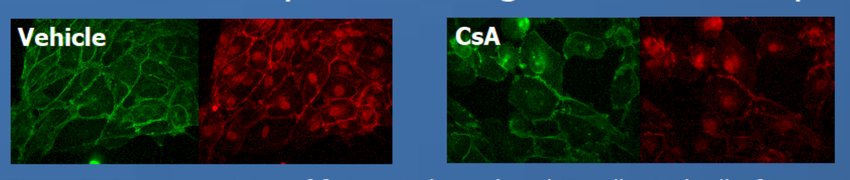


Figure 6: PTECS treated with CsA and TGFβ1 with and without type 1 TGFβ1 receptor inhibitor SB431542

RESULTS (4)

Despite the early reduction of K cadherin at the cell membrane and the apparent nuclear accumulation of its anchor protein β catenin (Figure 7), β catenin dependent transcription was not demonstrated by 24hrs using a Luciferase reporter assay.



400x Magnification

Figure 7: Immunostaining of β catenin (green) and K cadherin (red) after 4hrs CsA exposure showing apparent nuclear accumulation of β catenin

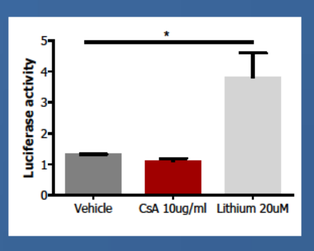


Figure 8: Luciferase Activity measured at 28hrs using a TCF/LEF reporter assay. Lithium was used for a positive control.

CONCLUSIONS

CsA induces a phenotypic change to primary proximal tubule cells. This may serve to contribute to renal fibrosis, independent to its effects on the vasculature.

The regulation of the adherens junction protein K cadherin, which decreases rapidly, may be a key initiating factor to this pathogenic change.

Despite many similarities to the phenotype induced by TGFβ1, these changes are independent of TGFβ1 and Wnt signalling suggesting an alternative secondary mediator.

THIS RESEARCH HAS BEEN SPONSORED BY THE SOUTH WEST KIDNEY FUND