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P456 -Dapagliflozin may attenuate renal fibrosis by inhibition of novel Smad3 linker region phosphorylation

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Introduction:

Diabetic Nephropathy (DN), driven by both transforming growth factor beta 1(TGFβ1) and hyperglycaemia, is the primary cause of chronic kidney disease. Increasing evidence implicates sodium/glucose cotransporter 2 (SGLT2) in mediating the effects of chronically elevated glucose on proximal tubule epithelial cells (PTECs). SGLT2 is the target of new anti-diabetic therapies collectively referred to as Gliflozins. We investigate whether the pro-fibrotic effect of raised glucose+TGFβ1 can be regulated by SGLT2 inhibition, before investigating the underlying cellular signalling pathways.

Materials /Methods:

Primary human PTECs were grown on collagen IV coated culture dishes. They were treated with D glucose at either 5.5 mM (normoglycaemic control), 25mM (hyperglycaemia) or 5.5mM D glucose+19.5mM L glucose (osmotic control), with/without TGFβ1 at 0.75ng/ml. The same cells were also administered Dapagliflozin (0.1μM, 1μM, 10μM). After time ranging from 5 minutes to 24 hours, cells were lysed. Heparin/agarose pull down was applied to the media to isolate heparin-binding proteins. Western blot was then used to detect the level of Connective Tissue Growth Factor (CTGF), EDA fibronectin, phosphorylated extracellular signal regulated kinase 2 (Erk 2), and phosphorylated Smad3 linker region serine 204 (LR s204) protein.

Results:

The upregulated CTGF protein secretion (mw 36-38kDa) observed in our 25mM D glucose+TGFβ1 treatment was significantly attenuated by Dapagliflozin at all three concentrations, reversing the expression back to approximately basal levels by 24h. TGFβ1± high glucose phosphorylated Erk 2 (mw 42kDa) from 5 to 60 minutes, while glucose treatment on its own phosphorylated Erk 2 from 15 minutes and is sustained up to 45 minutes. Control 5mM D glucose, 25mM D glucose, and TGFβ1 treatment did not show any significant activation of LR s204 (mw 38kDa) from 5 through to 60 minutes. On the other hand, there was a significant rise of LR activity observed at 30 minutes in our cells with 25mM D glucose+TGFβ1 when compared to the rest.

Discussion:

We have previously reported an optimised in vitro primary human PTEC model of DN in which a combination of TGFβ1 (0.75ng/ml) and D-glucose (25mM) significantly induced CTGF and EDA fibronectin secretion. Neither agonist alone was adequate to stimulate the fibrotic markers. Here we demonstrate a complete reduction of CTGF secretion by Dapagliflozin (0.1-10μM), implicating a key role for SGLT2. Both TGFβ1 and D-glucose separately produced an increase in cellular phospho-Erk, but there was no additive effect after co-incubation suggesting that the pro-fibrotic outcome is not dependent on direct Erk activation. However, we have identified a novel alternative mechanism by the aforementioned combined treatment; phosphorylation of a serine on the Smad3 linker region (LR). The canonical TGFβ1/Smad

pathway involves phosphorylation of the MH2 domain inducing nuclear localisation and gene transcription. Hayashida et al showed that glucose mediated Erk phosphorylation of LR potentiated Smad mediated transcription in mesangial cells [1]. Although we also show glucose induced LR phosphorylation in our PTECs, our data doesn't support a role for Erk.