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Dapagliflozin inhibits inflammatory and fibrotic responses in a human in vitro model of diabetic kidney disease

Dr Giulio Podda[1], Ms Xinlu Pan[1], Dr. Mark Dockrell[1]
[1]*South West Thames Institute for renal research, London, United Kingdom*

Background
Diabetic Kidney Disease (DKD) is the leading cause of end-stage renal failure worldwide. DKD is characterised by albuminuria and glycosuria leading to tubulointersitial fibrosis. Our group and others have demonstrated a role for fibronectin in its pathogenesis and as a biomarker. However, urinary inflammatory cytokines clearly also have a role as biomarkers in DKD; emphasising inflammation as part of the disease process. There is a direct correlation between urinary IL-18, albuminuria and albumin excretion rate, identifying its relationship with DKD. SGLT2 inhibitors have been shown to reduce morbidity and mortality in patients with DKD, consequently we have investigated whether they may act by limiting renal fibrosis and inflammation in a diabetic milieu. Using an in vitro model of DKD we have tested the potential for dapagliflozin to inhibit IL-18 and fibronectin expression in primary human proximal tubule cells.

Method
Primary human PTEC were cultured on collagen IV. To create a diabetic milieu cell were incubated with glucose at different concentration (5, 7 and 25 mMol) with and without TGFβ1, 0.7 ng/ml. PTEC were subsequently treated with dapagliflozin at increasing concentration (0.1 µl, 1 µl and 10 µl). After 24 hours cells were lysed and RNA extracted. Following RT QPCR was performed for, IL-18 and fibronectin. Relative expression was calculated by delta delta Ct with GAPDH as a housekeeping gene.

Result
Neither TGFβ1 nor raised glucose alone induced fibronectin expression. However, the combination of TGFβ1, 0.7 ng/ml and D-glucose 25mMol induced a 2.5 fold increase in fibronectin RNA ($P<0.001$). This was almost completely inhibited by 1µMol dapagliflozin ($P<0.001$).
In the case of IL-18 D-glucose, 25mMol did not significantly alter IL-18 expression, however TGFβ1, 0.7 ng/ml significantly reduced IL-18 expression ($P<0.01$).
In the presence of TGFβ1 25mMol D-glucose increased IL-18 expression ($p<0.05$). Dapagliflozin significantly inhibited this IL-18 expression, but only at 0.1µM. There was no significant effect of 1 or 10 µM dapagliflozin on TGFβ1/Glucose induce IL-18.
We subsequently investigated the effect of dapagliflozin in 7 and 5 mMol glucose. Although Dapagliflozin had no significant effect compared to TGFβ1 at the lower glucose concentrations, a consistent pattern emerged of increasing IL-18 expression with increasing dapagliflozin concentration.

Conlusion
TGFβ1 /glucose mediated fibronectin and IL-18 expression can be inhibited by dapagliflozin. However, the role of glucose appears less in IL-18 expression and the effect of dapagliflozin is maximal at 0.1µMol. Further increase in dapagliflozin has a negative effect, increasing IL-18. Presumably this is due to lowered intracellular Na inducing an inflammatory response