Protective Effects of Empagliflozin Against Experimentally-induced Renal Fibrosis

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Background

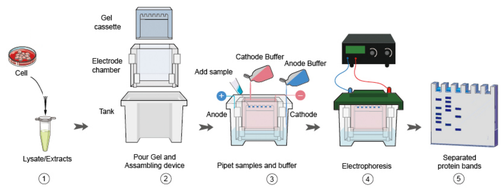
- Chronic kidney disease (CKD) is a growing epidemic worldwide with a global estimated prevalence of 13.4% [1].
- Hypertension, obesity, and diabetes are the leading risk factors for developing CKD.
- Sodium-glucose co-transporter 2 (SGLT-2) inhibitors have shown to be renoprotective in type 2 diabetes patients [2].
- The mechanisms that underlie the renoprotective effects of SGLT-2 inhibitors are unclear.
- Empagliflozin, an SGLT-2 inhibitor, has a higher selectivity for the SGLT-2 transporter than other drugs in the same class.

Study Objective

- To investigate whether empagliflozin treatment inhibits TGF-β-mediated fibrosis and pro-fibrotic signaling in renal proximal tubular cells.

Methods

- **Cell line:** NRK-52E (rat renal proximal tubular cells)
- **Treatment Groups**
  1. Vehicle-treated Control
  2. TGF-β-treated
  3. Empagliflozin-treated
  4. Empagliflozin-treated, TGF-β-treated
- **Treatment:** Vehicle or Empagliflozin (100 µM, 200 µM), for 48 hours
- **Western Blotting**



Fibrosis Markers

» Alpha-Smooth Muscle Actin (α-SMA)

» Vimentin

TGF-β Signaling Marker

» Phosphorylated SMAD 2

Results

1. Alpha-SMA Expression

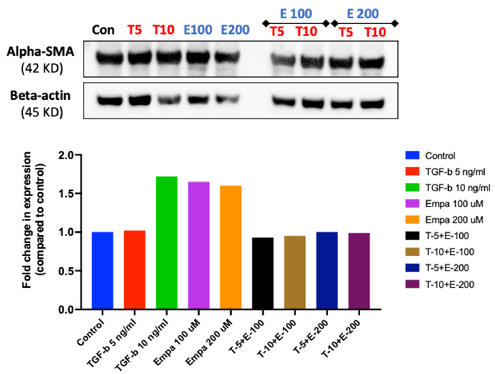


Figure 1. Representative Alpha-SMA expression over 48-hour period. Alpha-SMA expression normalized with beta-actin expression and densitometry data graphed below.

2. Vimentin Expression

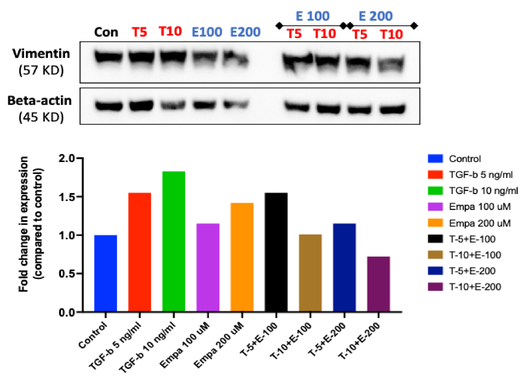


Figure 2. Representative Vimentin expression over 48-hour period. Vimentin expression normalized with beta-actin expression and densitometry data graphed below.

3. SMAD 2 Expression

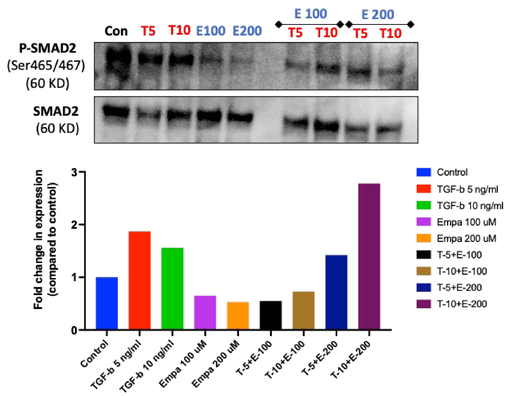


Figure 3. Representative phosphorylated and SMAD 2 levels over 48-hour period. SMAD 2 expression normalized with total SMAD 2 levels and densitometry data graphed below.

Summary & Conclusion

- 48-h TGF-β treatment induced fibrosis in NRK-52E cells
- 48-h Empagliflozin treatment (at 100 and 200 µM) - Inconsistent renoprotective effects
- Further studies are required to ascertain an effective concentration of empagliflozin to prevent renal fibrosis.

Future Studies

- Test empagliflozin at lower doses (100 and 500 nM).
- Determine whether empagliflozin decreases markers of -
  - Extracellular matrix proteins (Collagen IV, Fibronectin)
  - Inflammation (MCP-1, NLRP3)

Acknowledgments

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References

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2. Ravindran S, Munusamy S. Renoprotective mechanisms of sodium-glucose co-transporter 2 (SGLT2) inhibitors against the progression of diabetic kidney disease [published online ahead of print, 2021 Oct 29]. J Cell Physiol. 2021;10.1002/jcp.30621. doi:10.1002/jcp.30621

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