Nephroprotective effects of dapagliflozin in the adenine-diet induced mouse model of chronic kidney disease

Authors
Maria Ougaard[1], Louise Thisted[1], Grzegorz Maciag[1], Alex F. Hernandez[1], Frederikke Sembach[1], Henrik H Hansen[1], Stefan Tasler[2] Antje Meyer[2], Torsten Hoffmann[2], Michael Christensen[1]

[1]Gubra, Hørsholm Kongevej 11B, Hørsholm, Denmark
[2]Vivoryon Therapeutics N.V., Halle (Saale), Germany

Corresponding author
Michael Christensen: mch@gubra.dk

Background & Aim

Translational models are essential to identify improved treatment options for chronic kidney disease (CKD) patients. However, most preclinical CKD models do not demonstrate reduced glomerular filtration rate (GFR) or improvement by standard of care (SoC).

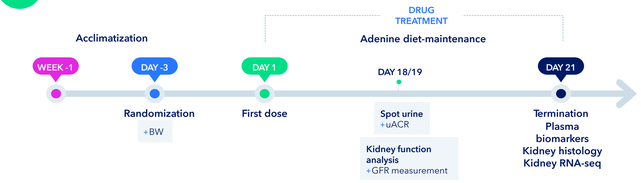
The aim of the present study was to characterize the functional, histological, and molecular effects of dapagliflozin in a short-term adenine diet induced (ADI) mouse model, thereby establishing a platform for more efficient profiling of potential novel therapies for CKD.

Methods

See study outline. Male C57BL/6JRj mice (11 weeks old) were randomized into study groups based on body weight. From day 1, control mice received control diet (S9352-E064, Ssniff, Germany), and adenine mice received 0.2% adenine (S9352-E060, Ssniff, Germany). Adenine diet-induced (ADI) mice were either treated with vehicle or Dapagliflozin (10 mg/kg, PO, QD or BID). At week 3, urine albumin-to-creatinine ratio (uACR) and GFR was measured and calculated based on $T_{1/2}$ of FITC-sinistrin clearance. Terminal plasma was collected for evaluation of urea and creatinine levels. The left kidney was weighed and processed for histomorphometric assessment of fibrosis (α-SMA, Col1a1, Col3a1), tubular injury (KIM-1) and macrophage infiltration (F4/80, CD11c).

www.gubra.dk

1 Study outline



Group	Animal	Gender	Number of animals	Treatment	Administration route	Dosing Frequency	Dosing volume	Dosing concentration
1	Control diet	Male	8	**Vehicle**	PO	Twice daily	5 ml/kg	-
2	Adenine diet (ADI)	Male	8	**Vehicle**	PO	Twice daily	5 ml/kg	-
3	Adenine diet (ADI)	Male	10	**Dapagliflozin QD**	PO	Once daily	5 ml/kg	**10 mg/kg**
4	Adenine diet (ADI)	Male	10	**Dapagliflozin BID**	PO	Twice daily	5 ml/kg	**10 mg/kg**

Figure 1. Study outline and group table

2 Dapagliflozin improves progressive weight loss

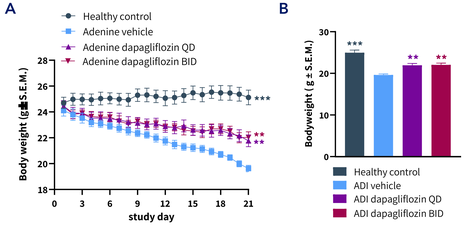


Figure 2. Dapagliflozin reverses progressive weight loss in ADI mice. (A) Body weight. **(B)** Terminal body weight. ***p<0.001 compared to ADI vehicle mice (Dunnett's test one-factor linear model).

3 Dapagliflozin improves kidney function and albuminuria

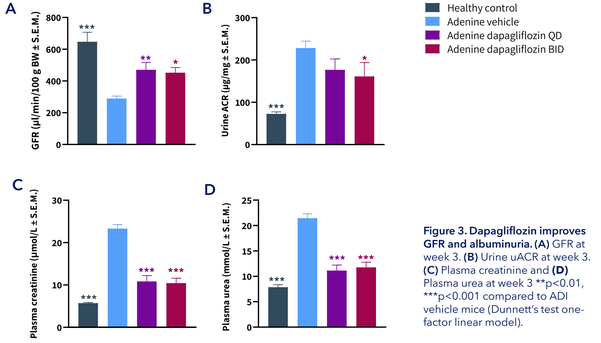


Figure 3. Dapagliflozin improves GFR and albuminuria. (A) GFR at week 3. **(B)** Urine uACR at week 3. **(C)** Plasma creatinine and **(D)** Plasma urea at week 3 **p<0.01, ***p<0.001 compared to ADI vehicle mice (Dunnett's test one-factor linear model).

4 Dapagliflozin reduces histological markers of kidney injury, inflammation and fibrosis

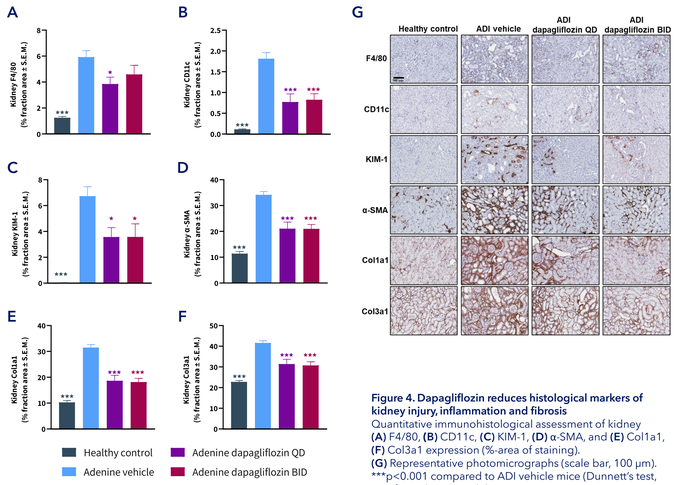


Figure 4. Dapagliflozin reduces histological markers of kidney injury, inflammation and fibrosis
Quantitative immunohistological assessment of kidney **(A)** F4/80, **(B)** CD11c, **(C)** KIM-1, **(D)** α-SMA, and **(E)** Col1a1, **(F)** Col3a1 expression (%-area of staining). **(G)** Representative photomicrographs (scale bar, 100 µm). ***p<0.001 compared to ADI vehicle mice (Dunnett's test, one-factor linear model).

5 Dapagliflozin shows therapeutic effects across renal disease markers

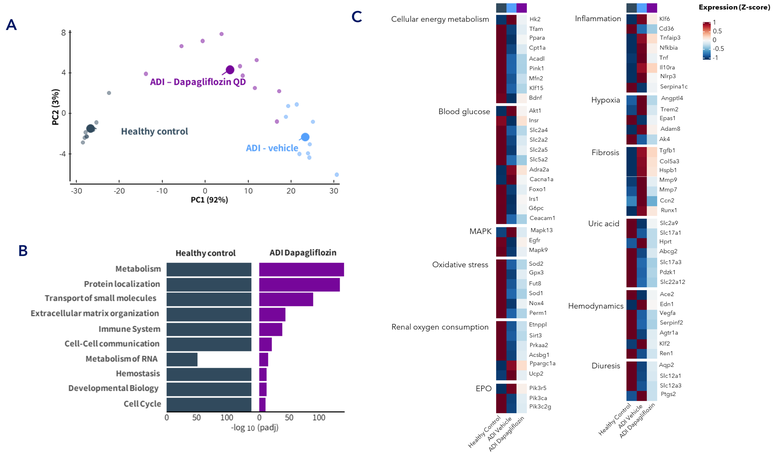


Figure 5. Dapagliflozin shows effects across renal disease markers. (A) Principal component analysis (PCA) plot of gene expression changes in top 500 most variable genes. Small dots indicate samples; large dots indicate the center of all samples. **(B)** Enriched Reactome pathways in Dapagliflozin treated ADI mice and healthy control mice vs vehicle-dosed ADI mice. Shown are inverse log10 p values of genes in the top 10 regulated pathways. **(C)** Curated list of gene expression markers reported to be implicated in CKD pathogenesis. Downregulated genes in blue and upregulated genes in red.

Conclusion

The present study in ADI mice establishes that dapagliflozin (QD and BID) similarly

+ Improves GFR and albuminuria
+ Reduces plasma creatinine and urea
+ Reduces tubular injury
+ Reduces renal inflammation and fibrosis

These findings support nephroprotective effects of dapagliflozin in CKD and highlights the applicability of the ADI mouse model in preclinical drug development.

Scan the QR code to learn more about our services within kidney diseases