Nephroprotective effects of standard of care in a state-of-the-art mouse model of hypertension-accelerated diabetic kidney disease

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BACKGROUND & AIM

Despite progress in treatment of diabetic kidney disease (DKD), drug discovery for DKD is challenged by the lack of animal models that display features of advanced human DKD.
AAV-mediated renin overexpression in diabetic, uninephrectomized *db/db* (db/db UNx ReninAAV) mice is a state-of-the-art model of hypertension-accelerated DKD with improved transability to human DKD (Østergaard et al., AJP Renal Physiol., 2021).
The present study aimed to evaluate standard of care (SoC) using combined angiotensin-converting-enzyme inhibitor (ACEi) and sodium-glucose cotransporter type 2 inhibitor (SGLT2i) treatment in the db/db UNx ReninAAV mouse.

METHODS

The study outline is depicted in Figure 1. Female *db/db* mice received a single intravenous dose of ReninAAV (2^{10} GC per mouse) in study week -5 and underwent unilateral nephrectomy (UNx) in study week -4. Prior to treatment, animals were randomized to treatment groups (n=16-18 per group) based on body weight and fed blood glucose measured in week -1. Mice received daily oral treatment for 12 weeks with vehicle, lisinopril (40 mg/kg) or lisinopril + empagliflozin (40+20 mg/kg). Endpoints included plasma and urine markers as well as kidney histopathology. Deep-learning computational analysis was applied for automated grading of glomerulosclerosis severity.

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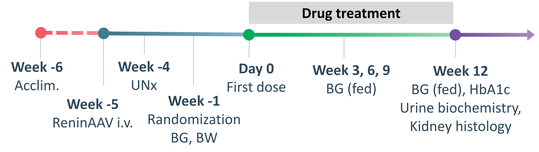
1 Study outline



Group	Animal	Gender	Number of animals	Treatment	Administration route	Dosing Frequency	Dosing volume	Dosing concentration
1	ReninAAV UNx db/db	Female	17	**Vehicle**	PO	QD	5 ml/kg	-
2	ReninAAV UNx db/db	Female	17	**Lisinopril**	PO	QD	5 ml/kg	**40 mg/kg**
3	ReninAAV UNx db/db	Female	17	**Lisinopril + Empagliflozin**	PO	QD	5 ml/kg	**40 mg/kg + 20 mg/kg**

2 Metabolic characteristics

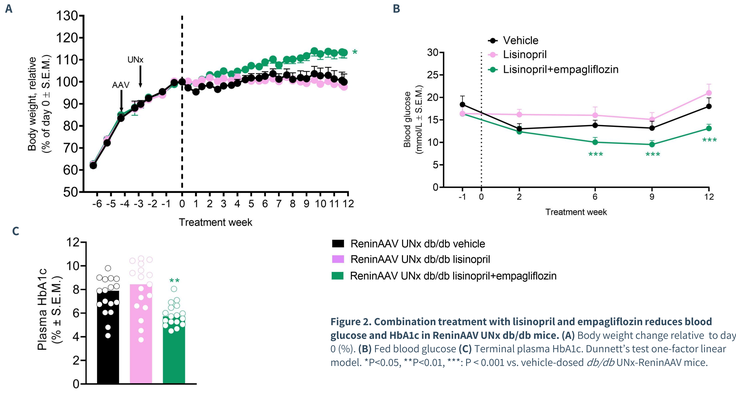
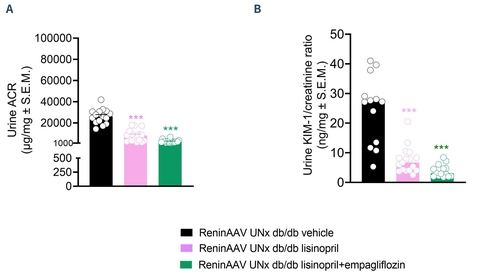


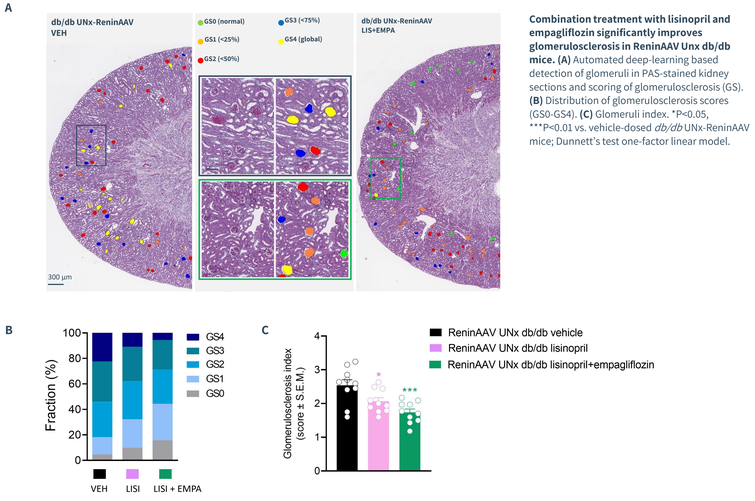
Figure 2. Combination treatment with lisinopril and empagliflozin reduces blood glucose and HbA1c in ReninAAV UNx db/db mice. (A) Body weight change relative to day 0 (%). **(B)** Fed blood glucose **(C)** Terminal plasma HbA1c. Dunnett's test one-factor linear model. *P<0.05, **P<0.01, ***: P < 0.001 vs. vehicle-dosed *db/db* UNx-ReninAAV mice.

3 Biochemical characteristics



Lisinopril and combination treatment with empagliflozin significantly improves urine markers of kidney injury in ReninAAV UNx db/db mice. (A) Urine albumin-to-creatinine ratio (ACR) at treatment week 12. **(B)** Urine KIM-to-creatine ratio at week 12 in the study. Data is presented as mean ± SEM (n = 17 per group). Dunnett's test one-factor linear model. ***P < 0.001 vs. vehicle-dosed *db/db* UNx-ReninAAV mice.

4 AI-assisted glomerulosclerosis scoring



Combination treatment with lisinopril and empagliflozin significantly improves glomerulosclerosis in ReninAAV Unx db/db mice. (A) Automated deep-learning based detection of glomeruli in PAS-stained kidney sections and scoring of glomerulosclerosis (GS). **(B)** Distribution of glomerulosclerosis scores (GS0-GS4). **(C)** Glomeruli index. *P<0.05, ***P<0.01 vs. vehicle-dosed *db/db* UNx-ReninAAV mice; Dunnett's test one-factor linear model.

5 Quantitative histological markers

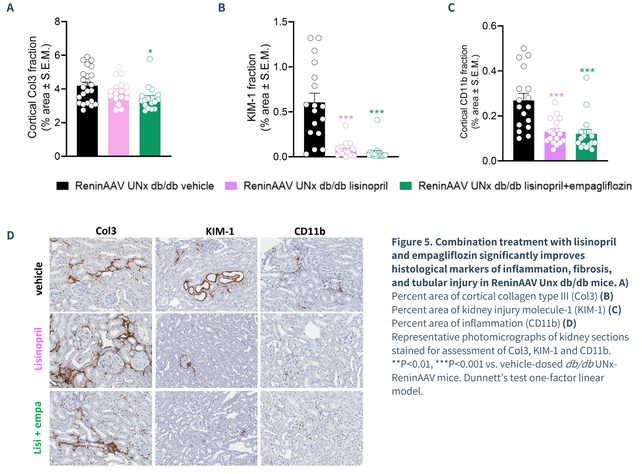


Figure 5. Combination treatment with lisinopril and empagliflozin significantly improves histological markers of inflammation, fibrosis, and tubular injury in ReninAAV Unx db/db mice. A) Percent area of cortical collagen type III (Col3) **(B)** Percent area of kidney injury molecule-1 (KIM-1) **(C)** Percent area of inflammation (CD11b) **(D)** Representative photomicrographs of kidney sections stained for assessment of Col3, KIM-1 and CD11b. **P<0.01, ***P<0.001 vs. vehicle-dosed *db/db* UNx-ReninAAV mice. Dunnett's test one-factor linear model.

CONCLUSION

Lisinopril treatment in db/db UNx ReninAAV mice:

+ Reduces albuminuria and urinary KIM-1 excretion
+ Reduces histological markers of inflammation, kidney injury and glomerulosclerosis

Lisinopril+empagliflozin treatment in db/db UNx ReninAAV mice:

+ Ameliorates body weight loss and improves glycemic control
+ Reduces albuminuria and urinary KIM-1 excretion
+ Promotes substantial improvements in glomerulosclerosis severity
+ Improves histological markers of kidney monocyte infiltration, fibrosis and tubular epithelial cell damage

Scan the QR code to see the paper: Therapeutic effects of lisinopril and empagliflozin in a mouse model of hypertension-accelerated diabetic kidney disease