Adriamycin-induced nephropathy (ADR) mouse model

-CRO service in Chronic kidney disease-

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Overview

1 Chronic kidney disease

2 ADR model: well-characterized disease model for CKD

3 Pharmacological study

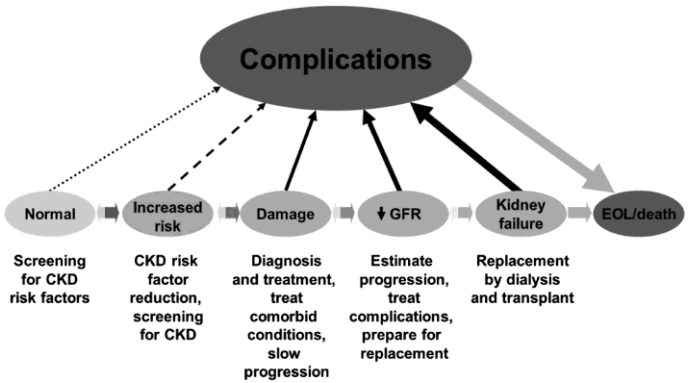
Chronic kidney disease

- Chronic kidney disease (CKD) is defined as **abnormalities of kidney structure or function** with implications for health. CKD is a worldwide public health problem, there is rising incidence and prevalence of kidney failure with poor outcome and high costs[1].
  - 15% of US adults — 37 million people — are estimated to have CKD.
  - 6.7% of annual budgets consumed by end stage renal disease (ESRD) patients.

Estimated CKD patients in US[1]

- Complications of CKD affect all organ systems[2].
  - Uremia, Cardiovascular disease, infection and impairments in physical function.
  - These complications are associated with higher morbidity and mortality.

- However, most of the treatments for CKD are symptomatic and limited.
  - Diagnosis in the early stages of kidney disease and the development of causal therapies is long required.



Conceptual model of CKD[2]

[1] https://www.cdc.gov/kidneydisease/pdf/2019_National-Chronic-Kidney-Disease-Fact-Sheet.pdf
[2] KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease

Key factors and endpoints in CKD progression

Prognosis of CKD by GFR and albuminuria category

GFR categories (ml/min/ 1.73 m²) Description and range				Persistent albuminuria categories Description and range		
				A1	A2	A3
				Normal to mildly increased	Moderately increased	Severely increased
				<30 mg/g <3 mg/mmol	30–300 mg/g 3–30 mg/mmol	>300 mg/g >30 mg/mmol
	G1	Normal or high	≥90		Monitor	Refer*
	G2	Mildly decreased	60–89		Monitor	Refer*
	G3a	Mildly to moderately decreased	45–59	Monitor	Monitor	Refer
	G3b	Moderately to severely decreased	30–44	Monitor	Monitor	Refer
	G4	Severely decreased	15–29	Refer*	Refer*	Refer
	G5	Kidney failure	<15	Refer	Refer	Refer

■ CKD is classified based on cause, GFR category, and albuminuria category (CGA)[1].

Clinical endpoints in CKD

End Point	Strength of Evidence
Kidney failure	Clinical outcome
Doubling of Scr (confirmed) (57% eGFR decline)	Valid surrogate end point
GFR decline > 40% (confirmed) GFR slope reduction (mean) > 0.5-1.0 mL/min/ 1.73 m^2/y	Valid surrogate end point
GFR decline > 30% (confirmed) UACR reduction (mean) > 30%	Reasonably likely surrogate end point in many trials and valid surrogate end point in some trials

Note: Hierarchy may vary depending on study population and trial design. Doubling of Scr and 30% to 40% decline in eGFR are used to determine response at the individual level; UACR reduction and GFR slope reduction are used as averages for comparing 2 or more groups.
Abbreviations: eGFR, estimated glomerular filtration rate; RCT, randomized controlled trial; Scr, serum creatinine; UACR, urinary albumin-creatinine ratio.

■ Key factors of CKD[2]:
- Proteinuria
- GFR
- Kidney biopsy (Histology)

[1] KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease
[2] Levey *et al.*, *Am J Kidney Dis.*,. 2020 (A Scientific Workshop by NKF, FDA and EMA)

Most treatments for CKD are symptomatic for preventing ESRD

Target	Treatment / Drug (e.g.)	Aim	Grade	
			S	E
Life style change	Dietary management Weight management Physical activity	Initial components of treatment and secondary prevention	**I**	**A**
Renin Angiotensin Aldosterone Blockade	ACE inhibitor ARB Aldosterone antagonist	Prevent the rate of progression to end-stage renal disease	**I**	**A**
Hypertension	Diuretics Calcium channel blocker Beta Blocker	Reduces renal disease progression and cardiovascular morbidity/mortality	**I**	**A**
Diabetes	Biguanide DPP-4 inhibitor	Decrease risk for cardiovascular disease and mortality	**I**	**A**
Dyslipidemia	Statin	Decrease the risk of cardiovascular or atherosclerotic events	**I**	**A**

Grading of Recommendations:
S (Strength of recommendation): **I** = generally should be performed
E (Evidence for recommendation): **A** = randomized controlled trials

Source: Management of Chronic Kidney Disease Guidelines for Clinical Care Ambulatory

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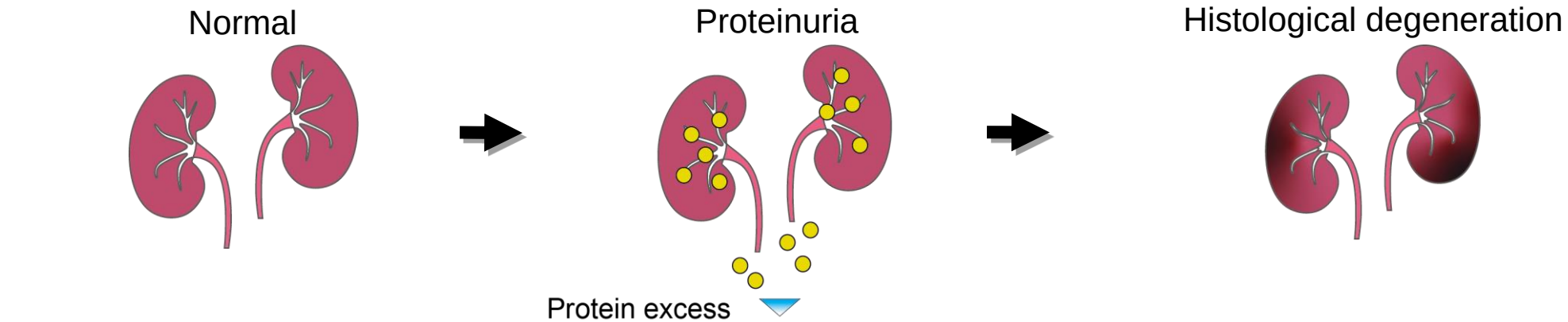
2 ADR model: well-characterized disease model for CKD

3 Pharmacological study

ADR model: well-characterized disease model for CKD

ADR model



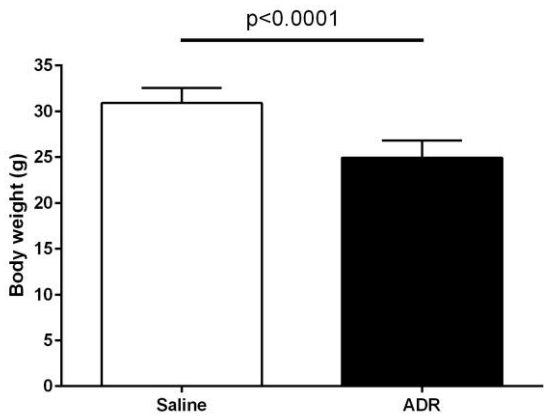
- The Adriamycin-induced nephropathy (ADR) model is a well-characterized disease model for chronic kidney disease and mirrors human CKD caused by primary focal segmental glomerulosclerosis (FSGS).

- The ADR model is characterized by podocyte injury followed by glomerulosclerosis, tubulointerstitial inflammation and fibrosis, which makes this model attractive for simple proof-of-concept studies for CKD, or in vivo screening for anti-fibrosis molecules [1].

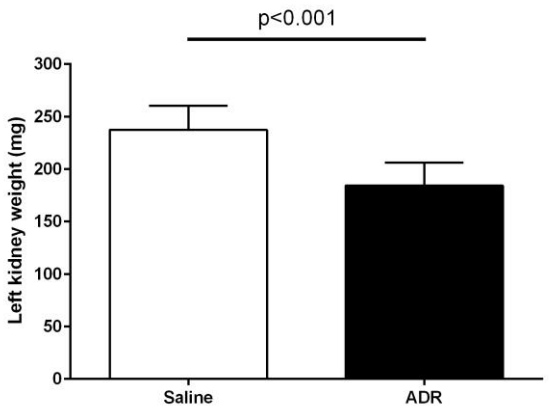
[1] Lee *et al.*, *Nephrology*., 2011

General parameters in ADR model mice (Day 28)

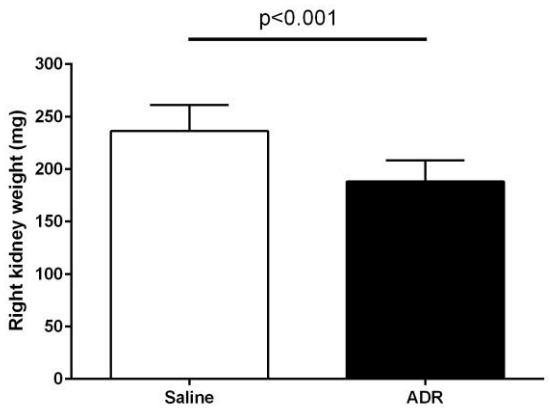
Body weight



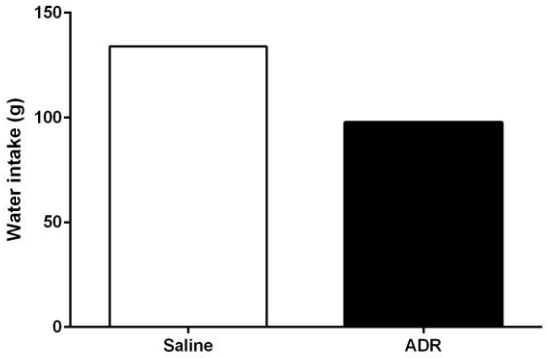
Left kidney



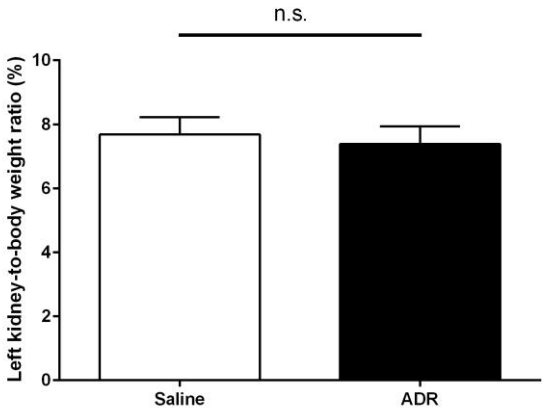
Right kidney



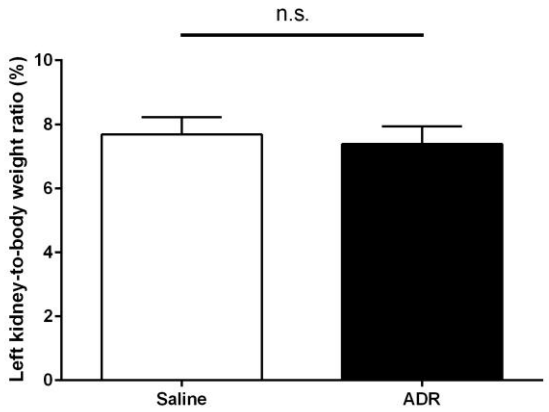
Water intake



Left kidney-to-body weight ratio



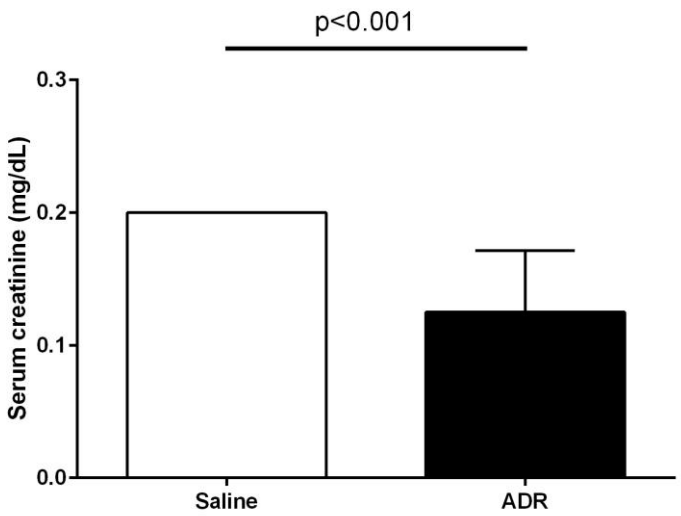
Right kidney-to-body weight ratio



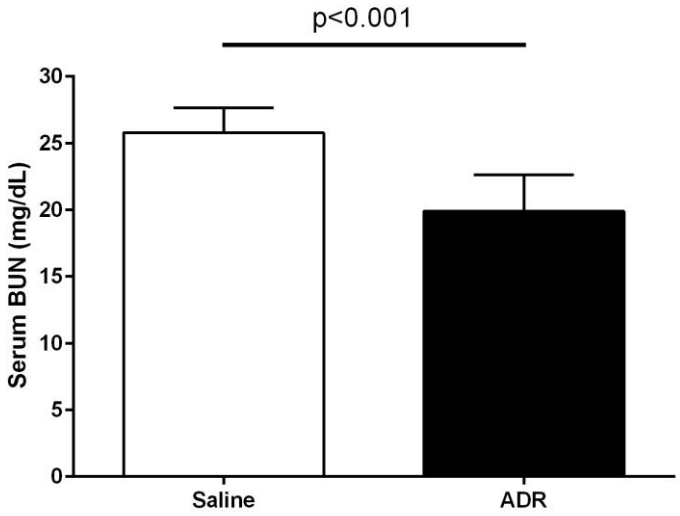
n=8 (Mean ± SD)

Serum biochemistry in ADR model mice (Day 28)

Serum creatinine



Serum BUN



n=8 (Mean ± SD)

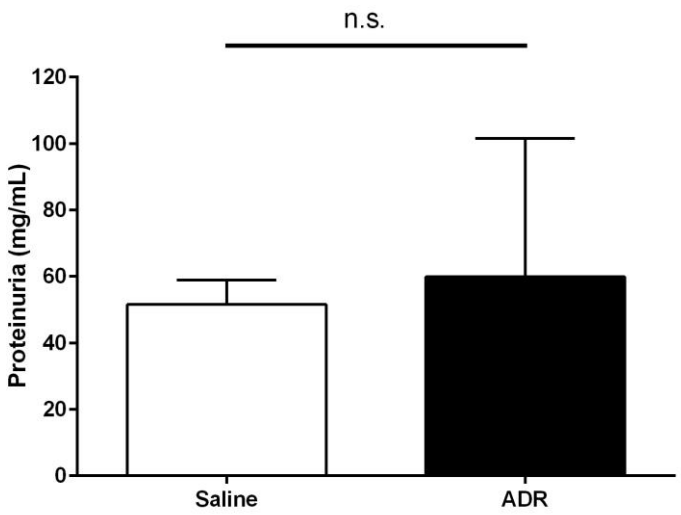
- Serum creatinine and BUN are decreased in the ADR model at day 28
- Serum BUN is significantly increased at a later stage[1]

[1] Caroline et al., *Caroline et al., Am J Physiol Renal Physiol*., 2010

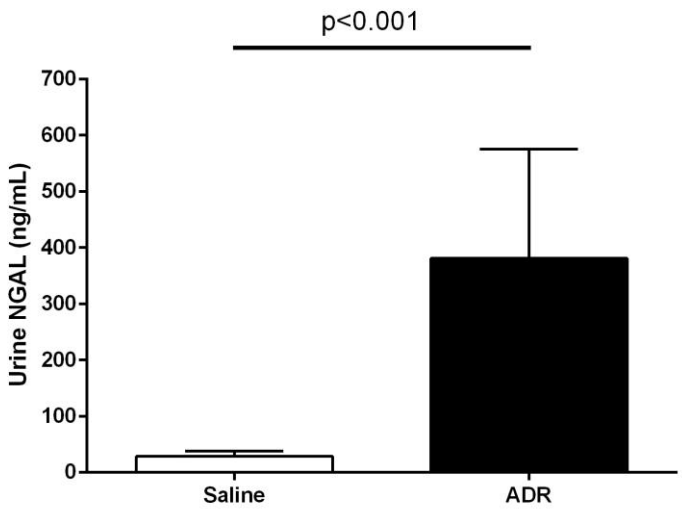
Urinal protein and biomarker in ADR model mice

Proteinuria and NGAL (Urinal biomarker correlated with progression of CKD)

Proteinuria (Day 7)



NGAL (Day 28)

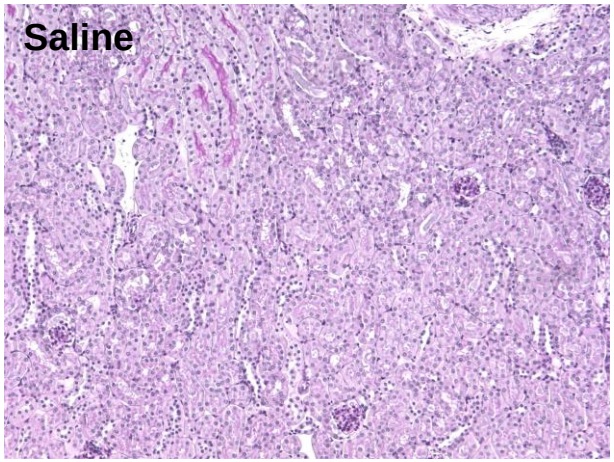


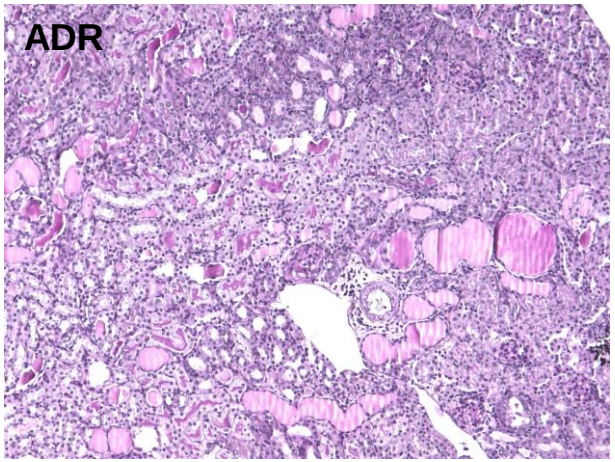
n=8 (Mean ± SD)

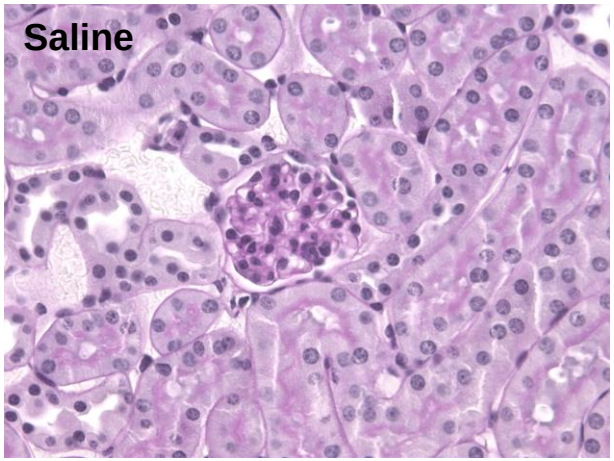
- No significant change in proteinuria in the ADR model at day 7, but starting to increase
- NGAL level is increased in the ADR model at day 28

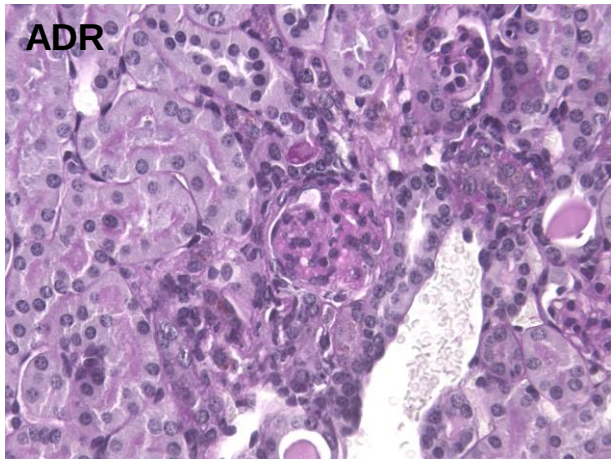
Histological parameter in ADR model mice (Day 28)

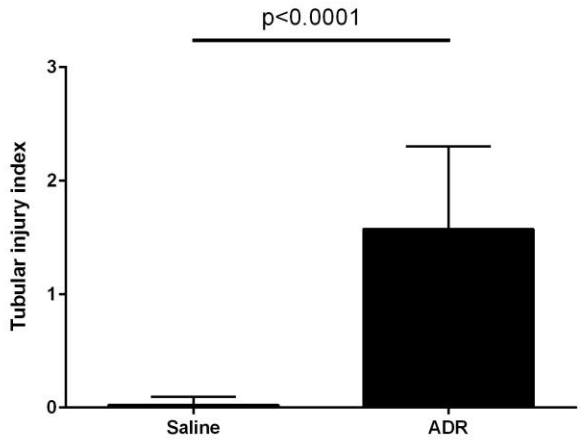
Representative microphotographs of PAS stained kidney sections

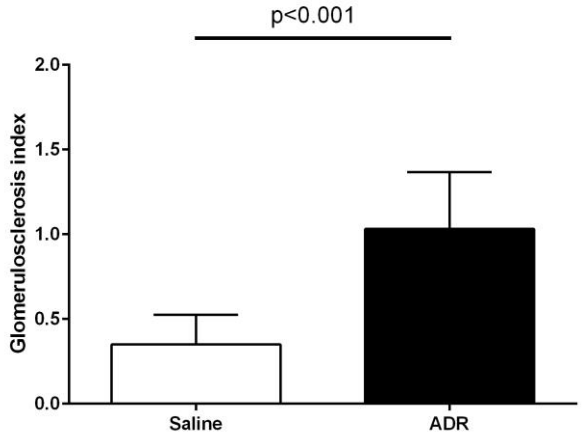












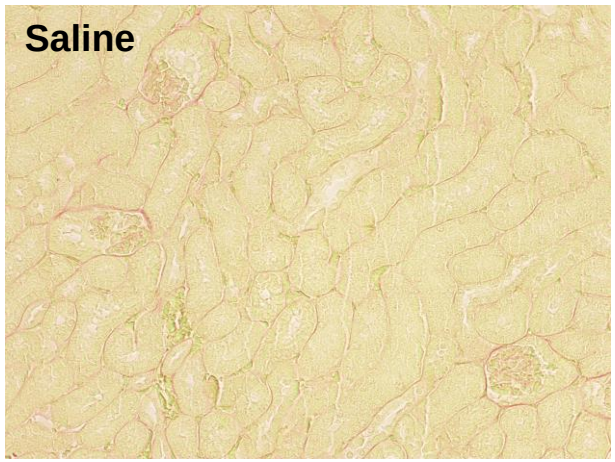
- Glomerular and tubular injury is observed at day 28, but not all glomerulus showed injury.

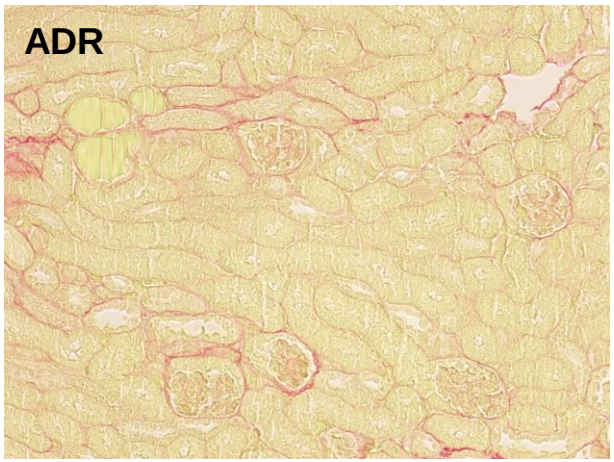
n=8 (Mean ± SD)

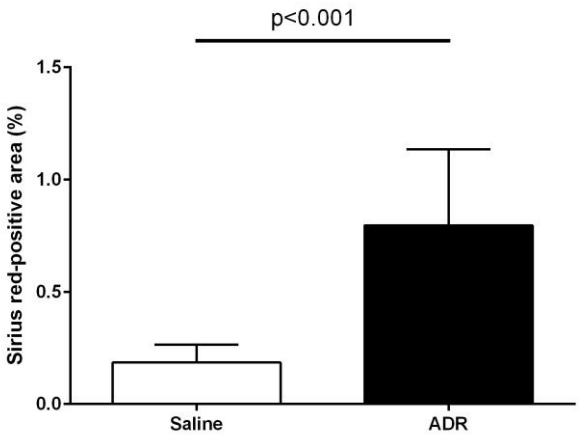
Original magnifications,
Upper panels, x100.
Lower panels, x400.

Fibrosis in ADR model mice (Day 28)

Representative microphotographs of SR stained kidney sections







n=8 (Mean ± SD)

Original Magnification: x100

- Collagen deposition is observed at day 28 in the ADR model and starts to increase after day 14.

Overview

1 Chronic kidney disease
2 ADR model: well-characterized disease model for CKD
3 Pharmacological study

Pharmacological study: Study design

Study aim: To investigate potential effects of VPA in the ADR model

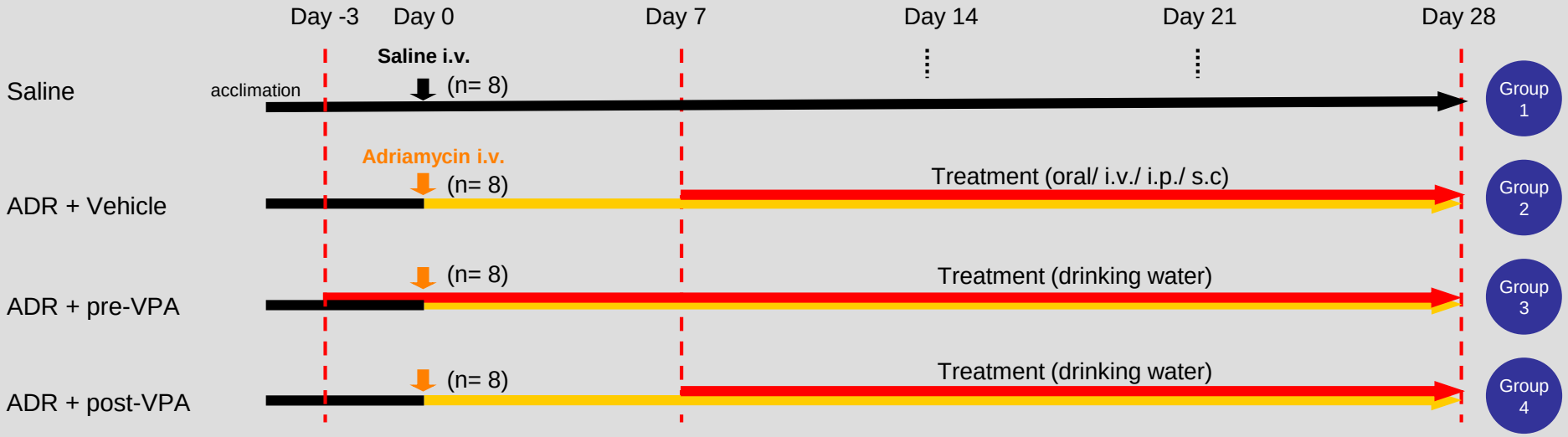
Rationale:

- Valproic Acid (VPA) is a histone deacetylase inhibitor and has anti-inflammatory and anti-fibrotic effects
- VPA attenuates proteinuria and kidney Injury [Beneden et al., *J Am Soc Nephrol*., 2011]

Clinical relevance of the study:

- VPA is being tested in Phase 2/3 clinical trials for FSGS [ClinicalTrials.gov]

Study design



Analyses

General

- Body weight
- kidney weight
- kidney-to-body weight ratio

Biochemistry

- Serum creatinine
- Serum BUN
- Proteinuria
- Urinal NGAL

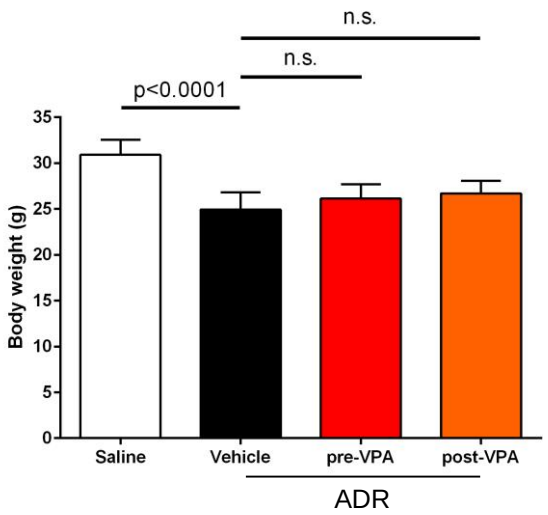
Histopathological assay

- PAS staining (glomerulosclerosis and tubular injury index)
- Sirius red staining (Fibrosis area)

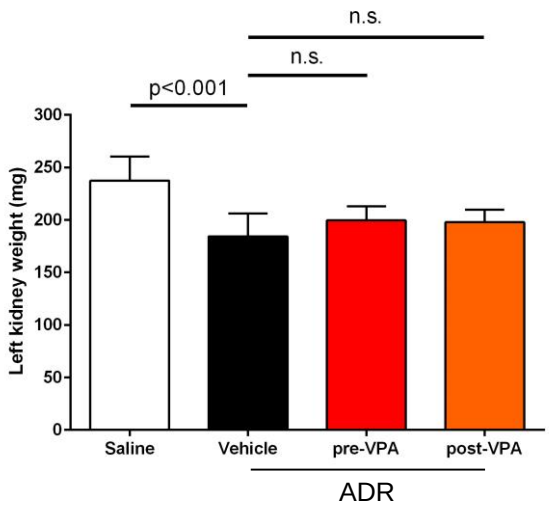
i.v.: intravenous administration

General parameters after VPA treatment (Day 28)

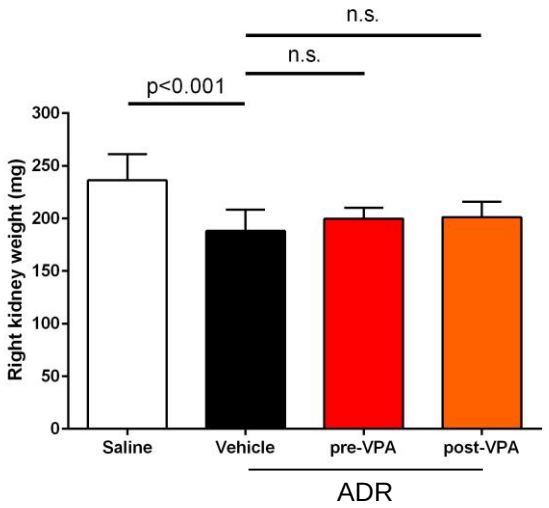
Body weight



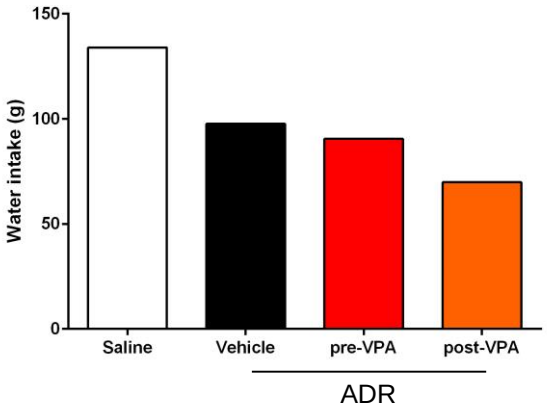
Left kidney



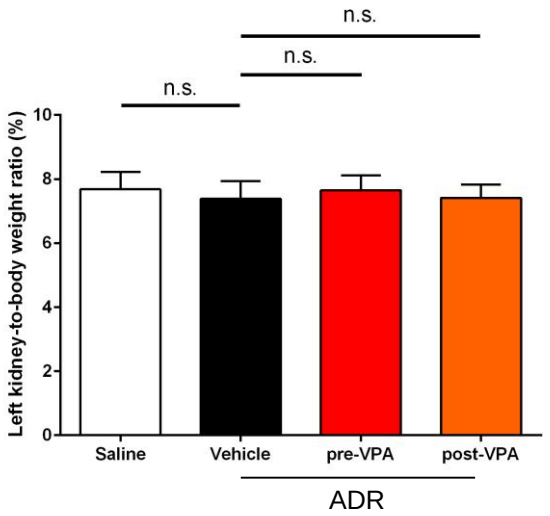
Right kidney



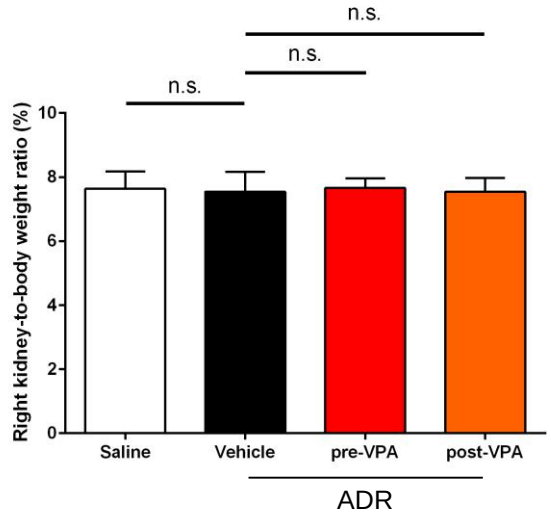
Water intake



Left kidney-to-body weight ratio



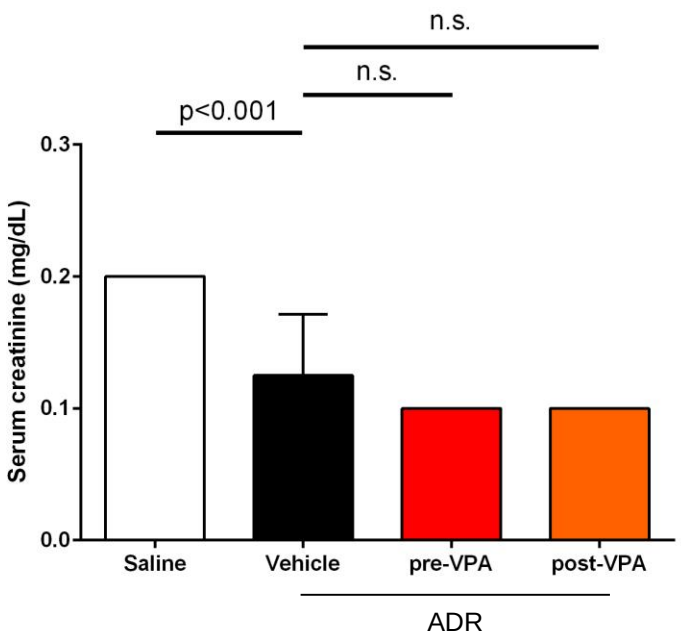
Right kidney-to-body weight ratio



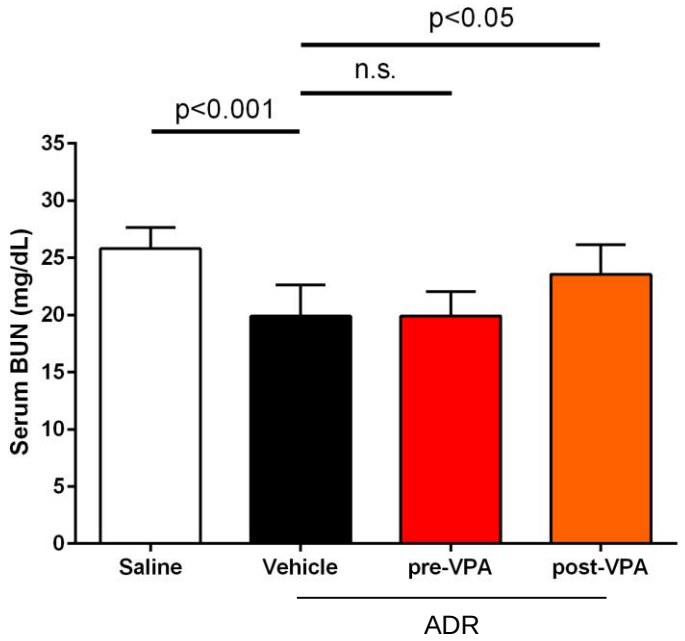
n=8 (Mean ± SD)

Serum biochemistry after VPA treatment (Day 28)

Serum creatinine



Serum BUN



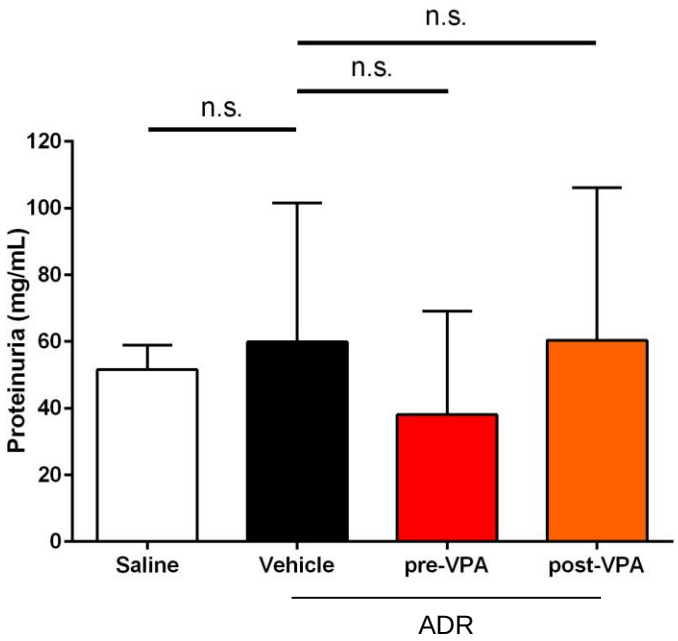
n=8 (Mean ± SD)

- VPA treatment did not show improvement of Serum biochemistry in the ADR model

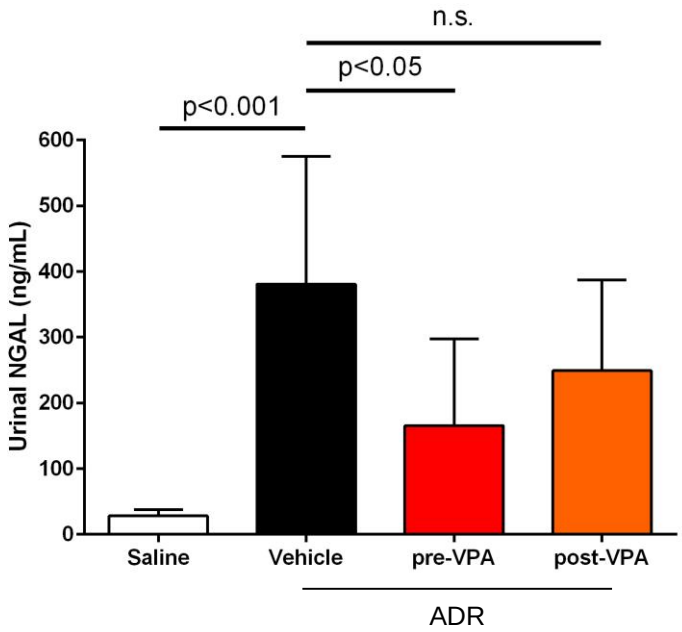
Urinal protein and biomarker after VPA treatment

Proteinuria and NGAL (Urinal biomarker correlated with progression of CKD)

Proteinuria (Day 7)



NGAL (Day 28)

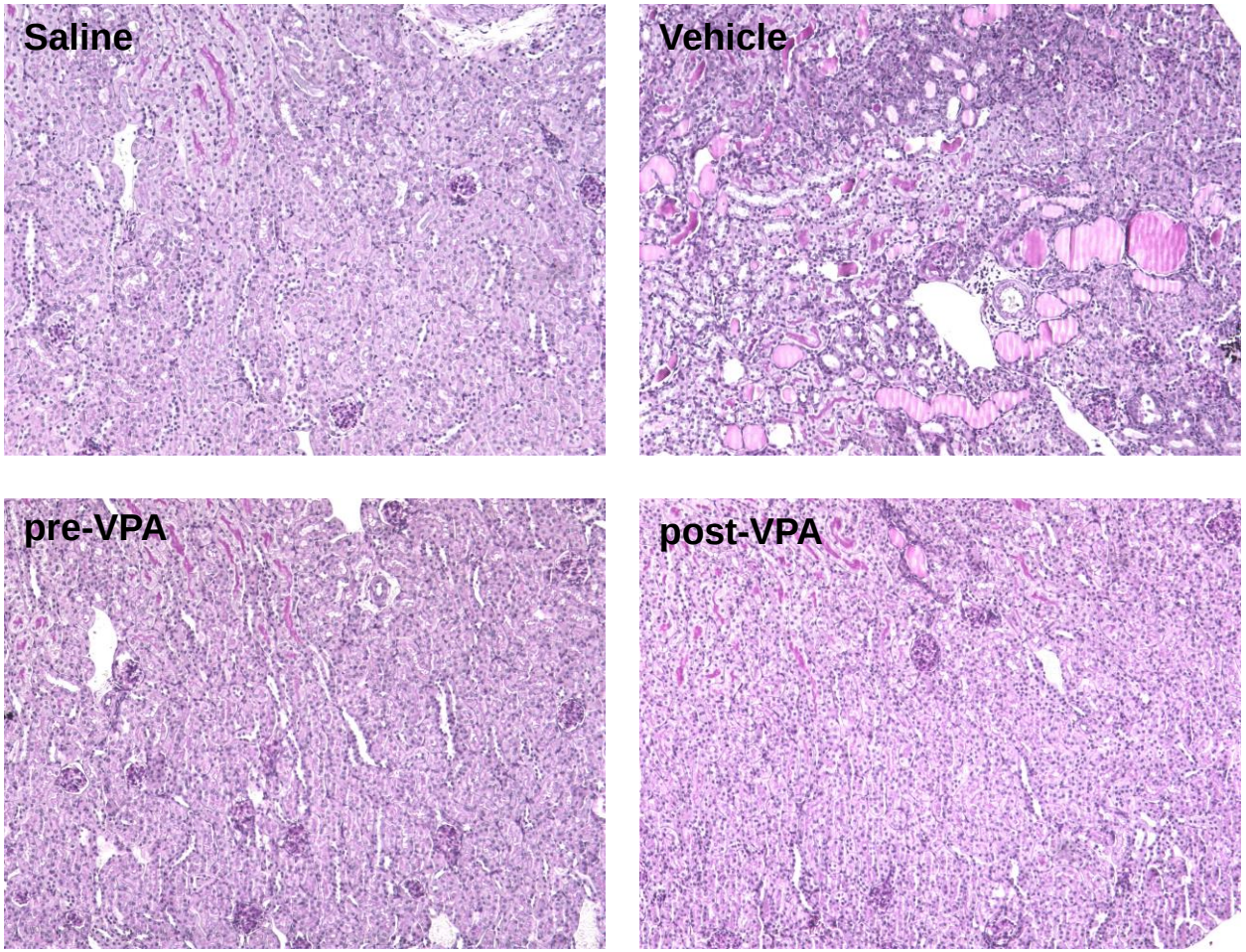


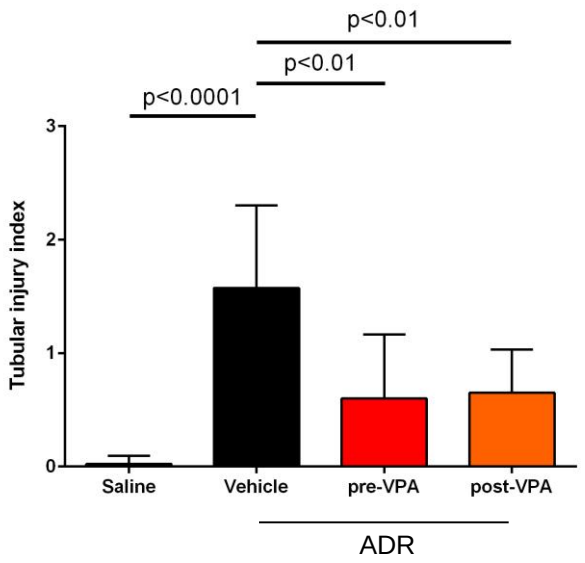
n=8 (Mean ± SD)

- Pre-VPA treatment showed a decreasing trend in proteinuria at day 7
- VPA treatment decreased NGAL level at day 28

Histological parameter after VPA treatment (Day 28)

Representative microphotographs of PAS stained kidney sections





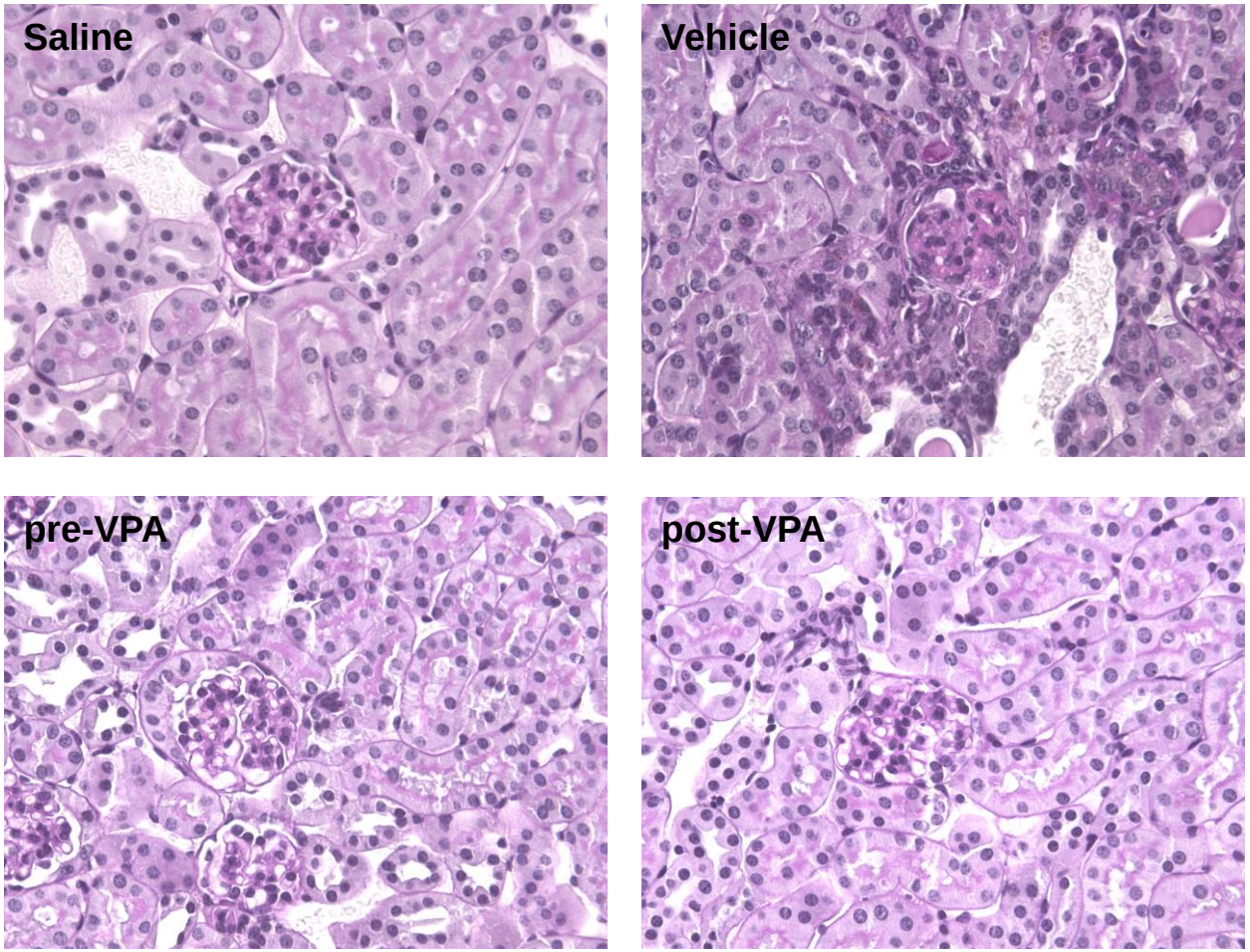
n=8 (Mean ± SD)

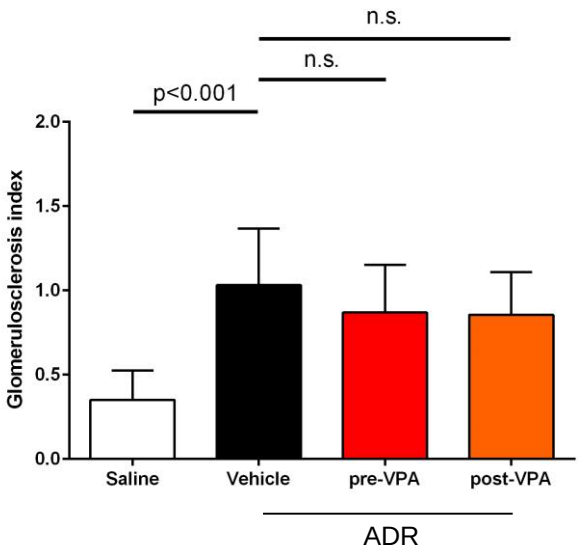
Original Magnification: x100

- VPA treatment showed significant decrease in tubular injury index at day 28

Histological parameter after VPA treatment (Day 28)

Representative microphotographs of PAS stained kidney sections





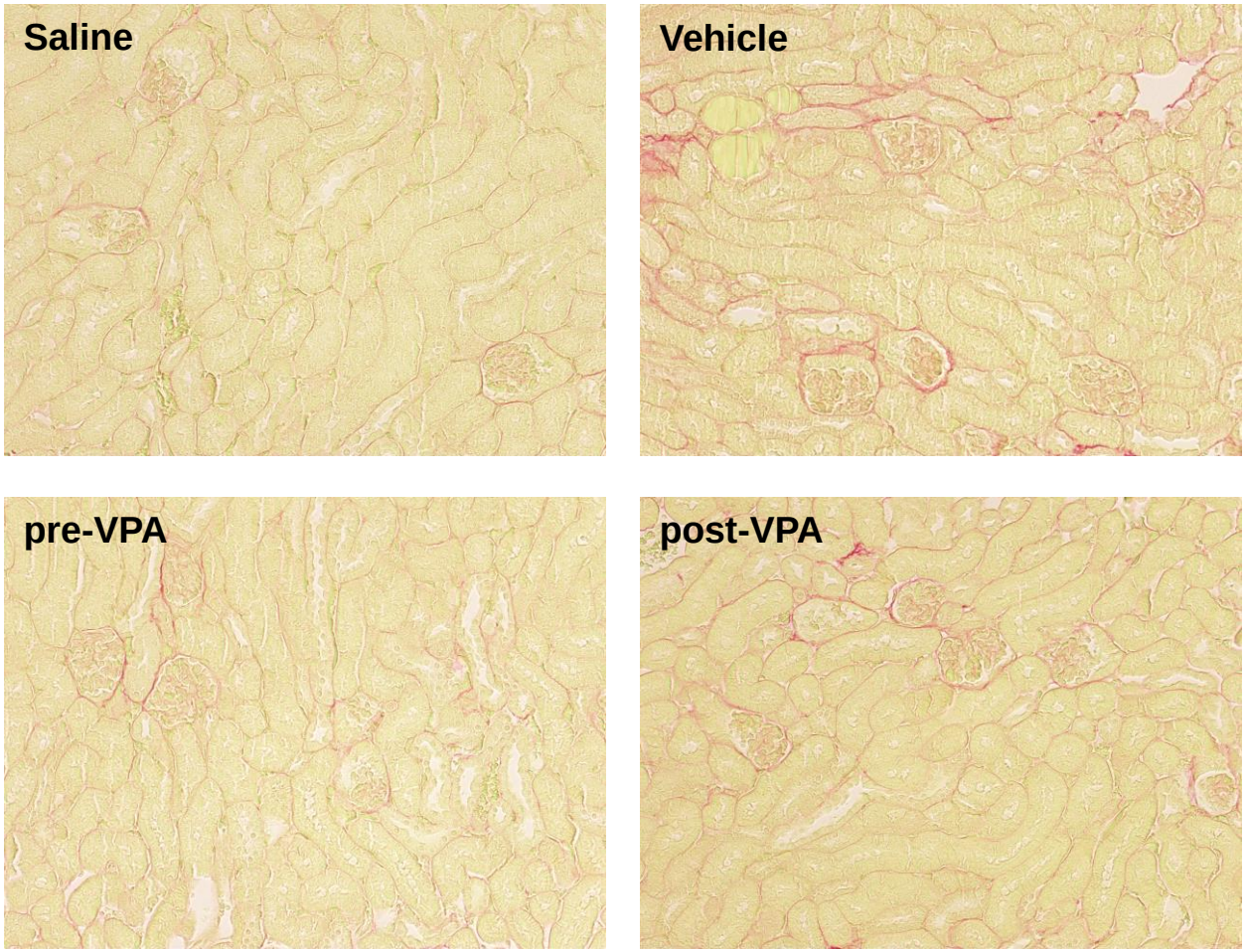
n=8 (Mean ± SD)

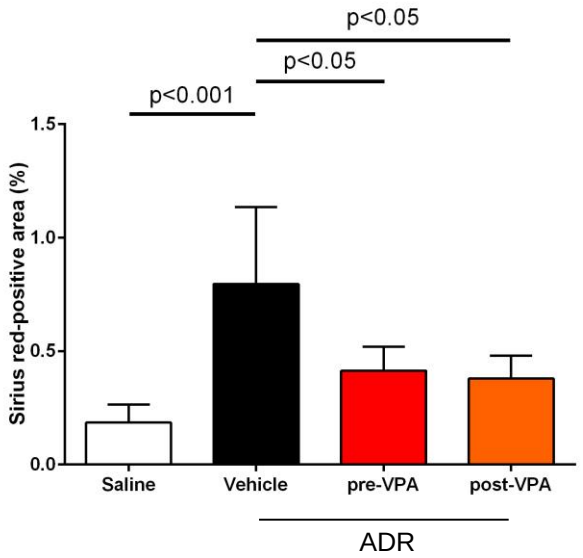
Original Magnification: x400

- VPA treatment did not show a significant decrease in glomerulosclerosis index at day 28
- However, number of high-score glomerulus are decreased by VPA treatment

Fibrosis after VPA treatment (Day 28)

Representative microphotographs of SR stained kidney sections





n=8 (Mean ± SD)

Original Magnification: x200

- VPA treatment showed significant decrease in Sirius red staining at day 28

Conclusion

- This model is appropriate for evaluation of early to mid disease progression of CKD.

Characteristics of ADR model

- Histological evaluation of glomerular and tubular injury, and fibrosis is possible.

- Proteinuria and NGAL evaluation are possible.
  - These parameters are more likely to be elevated early after kidney damage.

- Blood biochemistry markers are not increased.
  - However, increase in these parameters are shown only after a significant decrease of kidney function.

VPA treatment

- VPA treatment shows histological improvement in ADR model.

- VPA treatment shows Proteinuria and NGAL improvement.